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CURRENT CANCER RESEARCH

on

TUMOR-RELATED ANTIGENS

FEBRUARY 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service ● National Institutes of Health ●
National Cancer Institute

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This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 60 different cancer research areas are covered by a separate **SPECIAL LISTING.**

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

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CURRENT CANCER RESEARCH

I. ONCOFETAL ANTIGENS

A. CARCINOEMBRYONIC ANTIGEN

1. POSSIBLE ROLE OF CARCINOFETAL ANTIGENS IN THE IMMUNE RESPONSE OF THE CANCER PATIENT

VonKleist, S., Luckenbach, G. A., Pollack, M., Albert Ludwigs Universitat Freiburg im Breisgau, Inst. of Immunobiology, Dept. of Tumorimmunology, *Stefan Meierstr. 8, 7800 Freiburg im Breisgau, Federal Republic of Germany*

OBJECTIVE: To study whether the carcinofetal antigens we isolated previously from human colonic tumors, i.e., the CEA, NCA and MTA, play a role in humoral (or cellular) defense mechanisms in the patient.

APPROACH: 1. Patients' sera are screened for the presence of circulating antibodies directed against the isolated antigens by the passive hemagglutination technique. Pre- and post-operative titers are compared. 2. Frozen sections of tumors as well as culture tumor cells are incubated with positive sera and examined by the fluorescent technique. 3. Tumor membrane fractions are eluted and the eventually bound Ig's are analyzed for their antibody activity by several techniques, e.g., passive hemagglutination, immunofluorescence, precipitation, etc. 4. Patients' lymphocytes are tested as effector cells in an autologous system.

PROGRESS: Patients' sera have been shown to contain antibodies directed against NCA and MTA, but not CEA. These antibodies also fix to the tumor cell membrane in vivo and in vitro.

2. CARCINOEMBRYONIC ANTIGEN IN FECES OF NORMAL SUBJECTS AND PATENTS WITH CANCER OF THE COLON

Benitez Bribiesca, L., Perez Vega, E., Barrientos, F. J., Casas, I., Hospital de Oncologia, Division de Laboratorios, *AV. Cuauhtemoc 330, Mexico City 7, D.F., Mexico*

OBJECTIVE: To measure the levels of carcinoembryonic antigen (CEA) in feces of normal subjects and patients with cancer of the colon and determine whether these levels show an increase earlier and more consistently than plasma levels in patients. This would provide a convenient, reliable measurement of CEA levels for diagnosis of cancer of the colon.

APPROACH: CEA levels will be determined by means of RIA in feces and blood in a statistically significant number of smoker controls. The presence in serum of other oncofetal proteins known to be cancer markers will also be measured. CEA levels in serum and feces will then be determined in untreated patients, and the presence of tumor markers in serum will also be determined.

A standardized technique is being developed for the simple and reproducible determination of CEA levels in feces. In cancer patients the tumor size, degree of invasion, metastases, histologic type and length of development will be evaluated.

Once established, the fecal CEA values and presence of oncofetal protein in serum of normal subjects will be evaluated according to age, sex, and smoking habits and statistical average, standard deviation, t and p will be determined.

Results in cancer patients will be compared with normal values, with special attention to CEA levels. Values obtained in feces and serum will be compared to determine if there are significant increases in fecal CEA in patients and if so, whether this occurs earlier or more measureably in feces than in serum.

3. CORRELATION OF CEA CONCENTRATION IN THE SERUM AND IN TUMOR TISSUES WITH ITS HISTOCHEMICAL DEMONSTRABILITY IN TISSUE SECTIONS (ABBREV)

Bagshawe, K. D., Paradinas, F. J., Charing Cross Hospital, Dept. of Medical Oncology & Histopathology, *Fulham Palace Rd., London, W6 8RF, England, United Kingdom*

OBJECTIVE: To define precisely the localization of carcinoembroyonic antigen (CEA) and other tumor markers in surgical specimens of cancers of lung, breast, stomach, colon and rectum and to correlate this information with estimations of the concentration of the same antigens measured by radioimmunoassay on serum obtained preoperatively and by extraction from fragments of the same surgical specimens.

APPROACH: There is evidence from in vivo antibody localization studies that CEA may be more widely distributed in tumors than is suggested by elevated serum levels of this marker. Confirmation of this is important if the use of cell membrane markers for antibody targeting is to be widely applicable. Information on the distribution of CEA in various tumor types and on the concentration of CEA in tumor specimens in relation to serum concentration is limited. Frozen and conventionally fixed specimens will be used for tumor marker localization by immunofluorescence and immunoperoxidase techniques. Cells in suspension will be studied and morphometric studies on the tissue preparations performed with carcinoembryonic antigen measured in serum and tissue sections by double antibody radioimmunoassay.

PROGRESS: Project started in the summer of 1979.

4. CARCINOEMBRYONIC ANTIGEN STUDIES

Bagshawe, K. D., Charing Cross Hospital, Dept. of Medical Oncology, *Fulham Palace Rd., London, W6 8RF, England, United Kingdom*

OBJECTIVE: To assess the clinical usefulness of carcinoembryonic antigen (1) as a diagnostic aid in patients suspected of having a malignancy and (2) as a means of monitoring patients successfully treated for malignancy.

APPROACH: CEA is being investigated as a diagnostic aid in patients presenting with haematuria and suspected pancreatic disease. The usual diagnostic procedures are carried out and also a plasma specimen, for CEA determination, is taken. CEA is being investigated as an indicator of recurrent carcinoma in patients treated for bladder cancer and colorectal cancer. At each follow-up examination a plasma (and for bladder cancer patients urine) sample is taken for CEA determination. The results are being analyzed.

5. A STUDY OF THE NATURAL HISTORY OF COLORECTAL CANCER - CLINICAL STUDY OF TUMOR MARKERS IN COLORECTAL CANCER

Blumgart, L. H., Wood, C. B., Gillis, C. R., Bond, M., University of Glasgow, Royal Infirmary, Dept. of Surgery, *84 Castle St., Glasgow G4 0SF, Scotland, United Kingdom*

OBJECTIVE: To determine the clinical and practical value of two tumor markers, carcinoembryonic antigen (CEA) and pregnancy-associated alpha2 glycoprotein (alpha2PAG) in patients with colorectal cancer.

APPROACH: Serum CEA and alpha2PAG levels were measured preoperatively and at regular intervals postoperatively in patients with large bowel cancer. CEA was assayed using a radioimmunoassay double antibody technique. Alpha2PAG was assayed by an immunoassay method using the Laurell rocket technique. In the postoperative period, patients with two consecutively elevated CEA levels were submitted to a planned program of investigation for the detection of tumor recurrence.

PROGRESS. Of 404 patients who have been studied to date 199 underwent apparently curative surgery. In a follow-up period of 18-52 months, 40 patients developed two elevated CEA levels and tumor recurrence was confirmed in 37. Two patterns of CEA elevation have been noted in patients with tumor recurrence: a fast rise with grossly elevated CEA levels recorded within 6 months of the first recording of an elevated level, and a much slower pattern of rise with only moderate elevations in serum CEA levels within 12 months of the first recorded elevation. This is of prognostic significance in that those patients with a fast rise in CEA levels had a much poorer prognosis. Elevations in alpha2PAG levels corresponded to the clinical course of patients with colon cancer but compared less favorably with CEA as a tumor marker.

6. PLASMA DETERMINATION OF ASSOCIATED ANTIGENS TO PRIMARY ADENOCARCINOMAS

Bartorelli, A., Accinni, R., Cavalca, V., Biancardi, C., Ferrara, R., Bailo, M., Calderini, L., Macchi, P., Universita degli Studi di Milano, *Via C. Saldini 50, 20133 Milan, Italy*

The 125CEA-anti-CEA system, when employed on plasma in increasing concentrations, turns out to be able to detect more than 50% of clinically primary adenocarcinomas.

This RIA system is used to monitor the extraction of CEA cross-reacting antigens from breast primary carcinomas (breast cancer antigen(s), BCA). The antisera raised against these antigens, and the 125CEA-anti-BCA cross-system, show the immunological differences between BCA and CEA.

The RIA 125CEA-anti-BCA system, when clinically employed, substantiates the hypothesis of the presence of antigens, more or less cross-reacting with CEA in primary adenocarcinomas. Therefore, CEA should no longer be considered as a general adenocarcinoma marker, but as a glycoprotein, extracted from adenocarcinoma liver metastases, cross-reacting with similar glycoproteins from primary adenocarcinomas and, to a greater extent, from their metastases.

Thus the CEA and BCA experimental models allow only a diagnosis of early cancer, but not organ localization. The latter is theoretically impossible with glycoprotein (antigen) cross-reacting with CEA, even though, as BCA, extracted from primary adenocarcinomas. In fact they always cross-react with similar glycoproteins (antigens), from primary adenocarcinomas and obviously, from their metastases.

BIBLIOGRAPHIC REFERENCES: A. Bartorelli, R. Accinni; Current Trends in Tumor Immunology: 233-252, 1979. S. Orefice, A. Bartorelli; Current Trends in Tumor Immunology: 253-268, 1979.

7. IMMUNOLOGICAL DIAGNOSIS, PROGNOSIS, AND TREATMENT MONITORING OF PATIENTS WITH BENIGN AND MALIGNANT DIGESTIVE TRACT DISEASES

Zamcheck, N., Loewenstein, M. S., Rau, P., Kupchik, H. Z., Mallory Inst. of Pathology Foundation, Gastrointestinal Research Laboratory, *818 Harrison Ave., Boston, Massachusetts,* 02118, United States of America

Measurement of pancreatic juice CEA activity in conjunction with plasma assay levels may help in diagnosing pancreatic cancer and in distinguishing it from pancreatitis. However it is of limited clinical application. Furthermore, our findings to date suggest that the liver plays a key role in regulating circulating CEA levels, extracting the antigen from blood and excreting it, in a measurable amount, in the bile. Again, from the clinical point of view, obtaining bile for measurement of CEA is usually not practical. If measurement of duodenal juice CEA reflects biliary output, then it is potentially useful. Our preliminary studies give evidence that direct measurement of duodenal juice CEA activity may be of practical use in detecting increased CEA production by primary and metastatic cancers and excretion into the bile and/or pancreatic juice.

The purpose of this study is 1) to assess the practicality of obtaining duodenal aspirates for CEA analysis by employing a new technique for duodenal intubation, 2) to increase the potential usefulness of combining measurement of plasma levels and duodenal output for the early diagnosis of digestive tract malignancy, 3) to asses the usefulness of post-operative and pre-operative duodenal juice CEA levels in patients undergoing resection of colorectal cancer, as an index of the completeness of surgical resection, 4) to determine whether increases in duodenal CEA outputs occur prior to elevations in circulating levels, and 5) if so, whether measurement of sequential duodenal CEA outputs in patients with resected colorectal cancers will detect early recurrence, permitting earlier second-look surgery or initiation of changes in treatment.

8. ABO BLOOD GROUPS AND CARCINOEMBRYONIC ANTIGEN IN COLONIC CANCER

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Specimens from twenty additional patients with carcinoma of the colon were collected. These specimens included normal and tumor tissue for histologic observation and detection of blood group and CEA antigens by immunologic methods (specific red cell adherence, immunofluorescence and immunoperoxidase); fresh frozen normal and tumor tissue for glycosyl transferase enzyme content determination; and pre-operative and post-operative plasma and serum for CEA content and glycosyl transferase activity determination respectively. Saliva was collected from all patients for determination of secretor status. In addition to surgical cases, material from 10 autopsies was collected for determination of blood group antigen distribution in the gastrointestinal tract.

In 46 cases of colon carcinoma thus far examined, specific red cell adherence (SRCA) studies for the detection of corresponding ABH blood group antigens showed 17 positive tumors (37%). In five cases, normal tissue was positive with or without positivity on the adjacent tumor. Four of these five cases were located in the proximal colon or terminal ileum. This indicates that blood group activity in normal tissue may be confined, for the most part, to the proximal colon or above in the gastrointestinal tract. Confirmation will await autopsy studies of normal blood group antigen localization. Positive tumors were found in all areas of the colon and included patients of all blood groups.

The study to date shows fewer secretors than expected (61%). The antigen pattern on normal and tumor tissue thus far appears unrelated to secretor status.

Limited studies using immunoperoxidase and immunofluorescent techniques parallel findings by SRCA.

9. TUMOR MARKER METHODOLOGY AND CHARACTERIZATION BY MOLECULAR AND ULTRASTRUCTURAL METHODS

Strobel, P. L., Ewing, E. P., Hicklin, M. D., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, Bureau of Laboratories, *1600 Clifton Rd. N.E., Atlanta, Georgia,* 30333, United States of America

PROJECT DESCRIPTION: The goal of this project is to study the appearance and physiological reactions of serum cancer markers (CEA) and correlate this with the clinical state of the patient and the course of disease. Factors causing nonsensitivity and nonspecificity of cancer marker tests are an important diagnostic problem. Two approaches are now available with the reagents which we have previously prepared: (1) Refined serum measurements of marker levels and immune complexes of these markers by radioimmunoassays, immunoelectrophoresis, and rocket immunoelectrophoresis; and (2) transmission electron microscopy visualization of tumor markers and their immune complexes on S. aureus, leukocytes, Raji cells, and tumor cells by using negative staining, electrondense labels, and autoradiography.

10. PATHOLOGY OF THE DIGESTIVE TRACT MUCOUS MEMBRANE

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We are continuing to study the immunopathogenesis of human digestive tract cancer by focusing on biological markers produced by benign and malignant mucous membrane. We are improving the clinical use of serial CEA levels as an early index of tumor recurrence and as an indicator for second-look surgery (by adding serial circulating immune complex measurements), as a monitor of therapy and as an index of prognosis.

Our studies emphasize the key role of the liver in regulating plasma CEA as well as its clinical significance. We will elucidate the effect of chronic and acute viral hepatitis on CEA

levels, both prospectively and retrospectively, and test the hypothesis that non-A, non-B viral hepatitis may be transmitted via CEA-positive transfused blood.

We are studying the use of the immunoperoxidase and other immunohistochemical techniques for identifying and localizing CEA and other tumor antigens in tumorous tissues and in body fluids. These methods are useful both for basic understanding and for clinicopathological use.

We will continue to use malignant ascites and pleural effusions as a primary source for new tumor glycoproteins, by identifying, purifying, preparing antibodies to them and developing assays for assessing their clinical usefulness.

BIBLIOGRAPHIC REFERENCES: Saravis, C.A., Oh, S.K., Pusztaszeri, G., Doos, W., and Zamcheck, N.: Tissue Localization of Zinc Glycinate Marker and Carcinoembryonic Antigen by Immunofluorescence. I. Preparation of Antisera against the Zinc Glycinate Marker. J. Nat'l. Cancer Inst., 60:1371-1374, 1978.

11. DEVELOPMENT OF AN IMPROVED METHOD FOR MEASUREMENT OF CARCINOEMBRYONIC ANTIGEN (CEA) BASED ON IMMUNOCHEMILUMINESCENCE

Velan, B., Sinai, Y., Israel Inst. for Biological Research, Depts. of Biochemistry/Microbiology, *P.O. Box 19, Ness Ziona, Israel*

Clinical studies employing radioimmunoassays for the measurement of CEA have clearly shown that the serum levels of the tumor antigens are related to the diagnosis of colorectal cancers. Moreover they may be of importance in the monitoring of treatment and management of cancer patients. The significance of CEA immunoassays in the evaluation of gastrointestinal cancers is complicated by the molecular heterogeneity of the CEA, as well as by antigenic cross reactivity with components of normal tissue.

This project is undertaken with the aim of increasing the specificity of CEA assays as well as the development of a convenient method of CEA measurement bypassing problems of short life of the reagents. Attempts to increase assay specificity will be based on: a) Purification and preparation of standard CEA from human colon carcinoma cell lines. b) Studies on the immunochemical sites of the CEA molecule employing the standard CEA and trials to isolate fragments of the CEA molecule endowed with high specificity. An enzyme linked immunoassay will be developed utilizing the CEA standards of higher specificity and the chemiluminescent method.

Efficiency of the improved CEA assay will be evaluated by comparison with currently employed radioimmunoassays in clinical trials testing cancer patient sera.

12. PREPARATION OF AN ORIGINAL RIA METHOD FOR DETERMINING CEA OF ADENOCARCINOMA OF THE COLON IN PLASMA

Buraggi, G. L., Bombardieri, E., Ringhini, R., Rodari, A., Turrin, A., Clerici, L., Istituto Nazionale per lo Studio e la Cura dei Tumori, Dept. of Nuclear Medicine, *1 Via G Venezian, 20133 Milan, Italy*

This study intends to prepare a CEA radioimmunoassay using the glycoproteins isolated from the primitive tumor. The use of the hepatic tumor extract CEA limits the specificity of the commercially available methods.

This research is done on tumors of the colon and the rectum, but it is also possible to carry this research out on other kinds of epithelial tumors on the condition that these tumors can secrete CEA-like glycoproteins in cancer patients. The extraction from the primitive tumor has been carried out according to three different methods of extraction; each of them allows obtaining CEA fractions from variously located cells. The first extraction is done by means of perchloric acid; afterwards DEAE chromatography and purification are carried out by means of gel filtration. Such methods allow us to obtain the CEA of the whole neoplastic cell (CEA W). The other two methods of extraction are intended to select the fraction of glycoproteins that must be extracted. They are based on the assumption that CEA-like glycoproteins exist in cell cytoplasm (CEA S) and on the cell membrane (CEA M). Both methods begin with a centrifugation, the end-products of which undergo different passages: extraction of the supernatant in ConA chromatography (CEA S) or extraction with SDC followed by WGL chromatography (CEA M).

CEA W, CEA S and CEA M seem to show different chromatographic patterns. They also determine different inhibitions in the antigen-antibody systems commercially available (CEA extracted from hepatic metastasis-related antibody).

We are immunizing three groups of animals in order to obtain specific antibodies. It will then be possible to know whether such heterogeneity determines the choice of a particular kind of fraction or whether for diagnostic and practical purposes the use of a single fraction or the whole extraction pool will give the same result.

13. IDENTIFICATION OF CEA IN CYTOLOGY SPECIMENS

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Cytologic examination of exfoliated cells in body fluids is an extremely valuable method for the early detection of cancer. Nonetheless, there remains a large area of uncertainty in the diagnosis of cytologic preparations, especially of sputum, bronchial washings and urine, where cellular morphologic changes of early malignancy cannot be distinguished from changes due to inflammation and regeneration. The objectives of this study are to provide an immunocytologic technique to detect carcinoembryonic antigen (CEA) on exfoliated cells as an adjunct to the cytologic distinction between malignant and benign atypical changes. Using an antiserum to human CEA, the immunoperoxidase staining method yields a dark precipitate at sites containing CEA while preserving light microscopic cytologic detail in conventionally fixed smears, cytocentrifuge preparations and cell blocks. Prior studies of tissue sections and a pilot study of cytology cell blocks have shown a very high correlation between cell-bound CEA and proven malignancy, including carcinoma in situ, among lung, intestinal, ovarian and bladder neoplasms. The results of the CEA-immunocytology will be compared to subsequent biopsy and clinical follow-up data in a 3-year study. It is expected that many cytologies now diagnosed as 'suspicious' or 'atypical' will be more accurately evaluated, resulting in earlier detection of curable cancers.

14. ASSAY FOR CARCINOEMBRYONIC ANTIGEN IN HUMAN SERUM

Zamcheck, N., Boston City Hospital, Gastrointestinal Research Laboratory, *818 Harrison Ave., Boston, Massachusetts,* 02118, United States of America

This is part of a broader project. A summary of this subproject is not available.

15. ASSAY AND STRUCTURE OF CARCINOEMBRYONIC ANTIGENS

Todd, C. W., Shively, J. E., Clark, B. R., Pande, H., Gibson, D., City of Hope Medical Center, Division of Immunology, *1500 E. Duarte Rd., Duarte, California,* 91010, United States of America

The goal of this research is to increase the clinical utility of the CEA assay for diagnosis and monitoring of malignant disease, particularly cancer of the large bowel. The approach being used is to define CEA chemically and to identify distinctive portions of the molecule against which antisera can be prepared. A clinical study comparing the utility of CEA and TEX for the diagnosis and monitoring of various forms of cancer has been initiated. The finding that the carbohydrate groups may be removed from CEA by treatment with anhydrous hydrofluoric acid has provided us with a more homogeneous preparation for cleavage to peptide fragments, which are now being sequenced.

16. SELECTIVE RADIOIMMUNOTHERAPY OF COLON CANCER

Primus, F. J., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky,* 40506, United States of America

Radioantibodies to CEA have been found to successfully localize tumors in experimental systems of human tumors in animals and in man. The purpose of this project is to take advantage of this selective tumor localization for more specific radiation therapy, utilizing the principles of neutron capture radioimmunotherapy. This involves the design and synthesis of

boron-containing compounds capable of labeling immunoglobulins prepared against carcinoembryonic antigen (CEA) with the retention of immunoreactivity. The boron-labeled immunoglobulins will serve as a means of transporting and localizing boron in colon cancer. Tumor cell destruction will then be accomplished by the well-known ability of 10-5B to absorb thermal neutrons with production of short-range high-energy particles. This concept and method will be evaluated in the GW-39 colonic cancer system, which is a serially propagated human colon tumor in hamsters. Evaluation of the method will be made on the basis of: (1) Concentrations of 10B in the tumors as compared to other host tissues; (2) microdistribution of 10B in tumor; and (3) tumor regression and/or destruction. This study should serve as a basis for possible later clinical trials.

17. CHEMICAL AND IMMUNOLOGICAL STUDIES ON CARCINOEMBRYONIC ANTIGEN (CEA)

Hammarstrom, S., Stockholms Universitet, Dept. of Immunology, *Box 6409, S10405 Stockholm 50, Sweden*

OBJECTIVE: To improve the disease related specificity of the CEA-test and to understand the function of CEA and the molecular mechanisms responsible for its appearance in the tumor cell.

APPROACH: Purification and characterization of CEA (colorectal, lung and bladder cancer) and of CEA-related normal adult tissue components. Comparative chemical and immumological analysis of these antigens. Structural studies on CEA. Cleavage of CEA in order to obtain immunologically active fragments. Production of antibodies with restricted CEA-specificity by somatic cell hybridization. Methodological studies on radio- and enzyme immunoassays. Studies on the expression of CEA and related normal antigens on colon carcinoma cell lines.

PROGRESS: A new CEA-related adult tissue component, biliary glycoprotein I (BGPI), was isolated and characterized. BGPI (mol.wt. approximately 85,000) was also detected in serum from healthy individuals (mean value 700 ng/ml). Patients with hepatobiliary diseases have increased BGPI levels in serum. A large N-terminal fragment of CEA (equals CEA low, mol. wt. approximately 125,000) was isolated from liver metastases of colorectal cancer. 'Normal cross-reactive antigen' (NCA) was purified from normal spleen and lung and from tumor. No difference in chemical and immunological properties between NCA from these three sources was seen. NCA appears to be a differentiation antigen in neutrophile granulocytes. It was found at the myelocyte stage. Fragments of CEA with intact disulphide bridges were prepared by trypsin treatment of citraconylated CEA. Several of these fragments were inhibitory in CEA assays utilizing antibodies against protein determinants in CEA. A sensitive and specific radioimmunoassay for CEA was developed. NCA and BGPI at concentrations 10 to the 4th power times higher than those of CEA did not give measurable values. Preliminary evaluation on a small clinical material (approximately 500 sera) showed high cancer specificity of the test.

18. HUMAN TUMOR ANTIGENS - STUDIES OF CARCINOEMBRYONIC ANTIGEN

Gold, P., Krantz, M., McGill Cancer Research Centre, *3655 Drummond St., Montreal, Quebec*, H3G 1Y6, Canada

OBJECTIVE: Elucidation of the biochemical and immunological relationships between CEA and cross-reacting substances from normal and neoplastic tissues. Information obtained will be used to evaluate the potential for diagnostic assays of improved specificity.

APPROACH: Cross-reacting substances will be purified and compared with CEA by limited proteolysis. Individual fragments will be further characterized by peptide mapping and immunological analysis. Unique fragments will be used to raise antisera with more restricted specificity and clinical investigations will determine if these materials are of use as diagnostic and prognostic tools.

PROGRESS: A large number of CEA fragments have been produced by limited proteolysis, and partial characterization has been obtained for 12 of the digestion products.

19. HUMAN TUMOR ANTIGENS - MONOCLONAL ANTIBODIES TO CARCINOEMBRYONIC ANTIGEN

Gold, P., Fuks, A., McGill Cancer Research Centre, *3655 Drummond St., Montreal, Quebec*, H3G 1Y6, Canada

The specific objective of this project is to develop monoclonal antibodies to carcinoembryonic antigen (CEA) and to use these in the immunochemical mapping of CEA as well as in the clinically utilized radioimmunoassay. The approach being used is the 'lymphocyte hybridoma' technique which involves fusion of splenocytes from immunized mice with a myeloma cell line. We are also exploring the application of this technique to the study of other human tumor antigens.

Early data indicate that a number of active hybridomas have been generated and cloning is under way.

20. STUDIES ON DISTRIBUTION OF ANTIBODY IN ATHYMIC NUDE MICE CARRYING HUMAN TUMORS WITH DEFINED ANTIGENIC MARKERS

Bagshawe, K. D., Lewis, J. C., Charing Cross Hospital, Dept. of Medical Oncology, *Fulham Palace Rd., London, W6 8RF, England, United Kingdom*

OBJECTIVE: To determine the distribution in body tissues and body fluids (and, wherever possible, the concentration) of heterospecific antibody directed at well-characterized antigens in tumor cell membranes.

APPROACH: Human gastrointestinal tract cancers which are known to synthesize the carcinoembryonic antigen are being grown in athymic nude mice. Highly purified gammaglobulin from animals immunized with CEA will be injected intravenously into the tumor carrying nudes. Body fluids, including extracellular fluid from normal sites and tumor sites, are being collected at various stages after intravenous administration to determine the concentration of antibody at these sites. Antibody concentration will also be determined by tissue excision and appropriate counting methods. Attempts will be made to determine the nature of antibody binding to tumor cells, in particular, whether this is specific or non-specific in nature.

Similar antibody coupled to potential cytotoxic agents will be similarly studied.

21. STUDY ON REACTIONS OF TUMOR ANTIGENS WITH THEIR ANTIBODIES

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This is ongoing research with a major objective of developing immunodiagnostic and immunotherapeutic procedures in malignancies. Considerable effort has been devoted to studies on carcinoembryonic antigen (CEA). The diagnosis of tumor based on demonstration of this antigen in feces has been, however, complicated by the appearance of CEA in normal GI mucosa and in food products. Immunization of rats and mice with an antigen analogous to human CEA is being energetically pursued. The appearance of newly discovered pancreas-specific antigen in duodenal contents and feces is being studied as a possible tool for the early diagnosis of pancreatic carcinoma. Formation of antibodies directed against tumor-specific cell-surface antigens (TSCSA) has been studied by means of several sensitive procedures, and diagnostic application of detection of such antibodies is being explored. Studies on genetic control of immune responses to TSCSA have been initiated. Special attention is devoted to antigens shared by tumor cells and normal allogeneic and xenogeneic cells. This includes extensive studies on heterophile antigens, primarily Paul-Bunnell antigen. This antigen was shown to appear not only in the course of infectious mononucleosis, but also in lymphoma and leukemia. The immune response to this antigen appears to be of great importance in the elimination of transformed, aberrant cells. Adsorption by tumor cells of erythrocytes sensitized by IgG antibodies is being explored as an intriguing biological phenomenon and a possible diagnostic procedure. Tumor growth-promoting activity of circulating immune complexes unrelated to the tumor antigens is also being investigated.

22. IMMUNOCHEMICAL STUDIES OF GASTROINTESTINAL CANCER

Alpert, E. E., Massachusetts General Hospital, Medical Services, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

In recent years it has become apparent that the detection and measurement of tumor-specific or tumor-associated antigens in the serum of cancer patients represents a new and exciting

approach to the immunodiagnosis of human malignancy. Experience in the last few years with radioimmunoassays specific for three tumor-associated carcinofetal antigens, alpha-1-fetoprotein (AFP), beta-fetoprotein or ferritin, and carcinoembryonic antigen (CEA), has demonstrated these markers to be of definite, although limited, diagnostic or prognostic usefulness. In addition to these carcinofetal proteins, there is increasing evidence that sera from malignant patients contain immune complexes which may contain new membrane- or tumor-cell-associated antigens. Therefore, our overall objectives in the next grant period will be (1) to measure these immune complexes (using several techniques) in the sera of patients with gastrointestinal malignancies and relate the level of immune complexes to the degree of disease; (2) to isolate these immune complexes, characterize their components and compare the components of the complexes in different GI malignancies; and 3) to develop radioimmunoassays to detect these newly shed neoantigens in the earlier stage of disease.

23. FUNDAMENTAL ENZYMOLOGIC STUDIES APPLIED TO ONCOLOGY

Fishman, W. H., La Jolla Cancer Research Foundation, *417 Coast Blvd. S., Box 1376, La Jolla, California*, 92037, United States of America

We hope to broaden our understanding of the carcinoplacental antigen, the Regan isoenzyme, as it relates to anomalous gene expression in human cancer. This phenomenon is part of the picture which includes 'fetal antigens' and ectopic polypeptide hormone production by human cancer. We plan to extend our work on Regan isoenzyme, to begin work on tumor non-Regan isoenzymes and to examine representative phosphohydrolases, such as acid phosphatase, as potential carcinoplacental antigens. It now appears that we have successfully cloned out two HeLa cell lines, one of which produces only Regan isoenzyme and the other, only non-Regan isoenzyme. Once these cell lines have been properly characterized and evaluated, we will be in a position to investigate known inducers, such as hydrocortisone and prednisone, inhibitors of DNA synthesis (hydroxyurea), and of DNA-directed RNA synthesis (Actinomycin D). Translational inhibitors such as puromycin, cycloheximide, chloramphenicol and BUdR will be included. Prior to this, the kinetics of the cell cycles of these two cell lines would be completed as a basis for all the inhibitor studies. For the better separation and study of Regan and non-Regan isoenzymes, we are developing a new method for separating isoenzymes based on the use of amino acid inhibitor Sepharose 4B column chromatography. Coupled with this are attempts to modify existing isoelectric focusing methodology. Finally, ultrastructural studies are aimed at specifically localizing isoenzymes of phosphohydrolases in HeLa cells and tumor tissue to provide a better basis for interpretation.

24. IMMUNOLOGICAL HETEROGENEITY OF CEA

Primus, F. J., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

The general objective is to identify and isolate antigenic variants of CEA and related glycoproteins of the human digestive system, and to investigate their physicochemical, immunological, and diagnostic properties. CEA and CCA-II are purified from liver metastases of colon adenocarcinoma by perchoric acid extraction and fractionation by molecular sieve, ion-exchange, and lectin and antibody affinity chromatography. CCA-III is purified from tumors and normal spleen by similar procedures. Goat and rabbit antisera raised against these antigens are used for the development by affinity chromatography of antibodies possessing different CEA, CCA-II, and CCA-III immunological specificities. Antigen species bearing these determinants in plasma and tissue specimens from normal individuals and patients with malignant and non-malignant gastrointestinal disease will be studied by radioassay, immunohistochemical, and physicochemical procedures.

25. CARCINOEMBRYONIC (CEA) ANTIGEN (HUMAN)

Schwartz, M. K., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

26. CEA AND OTHER TUMOR-ASSOCIATED SUBSTANCES IN BLOOD

Schwartz, M. K., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

27. BIOSYNTHESIS OF CARCINOEMBRYONIC ANTIGEN

Mendicino, J. F., University of Georgia, Franklin College of Arts & Sciences, Dept. of Biochemistry, *Athens, Georgia*, 30601, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

B. ALPHA-FETOPROTEINS AND OTHER ONCOFETAL ANTIGENS

28. THE VALUE OF SERUM AFP IN ASSAY IN DETECTING LIVER REGENERATION AND NEOPLASTIC TRANSFORMATION IN CHRONIC LIVER DISEASE (ABBREV)

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Moderately high values of serum alpha-fetoprotein (AFP) are encountered in a significant number of chronic liver disease cases.

At a basic level, the serum AFP value could be representative of the intensity of the regeneration of liver parenchyma. Alternatively, it may reflect merely the rate of dedifferentiation (not directly connected with the overall regeneration). However, subjects developing neoplasia may display a different pattern of evolution of the serum AFP concentration. If this proves correct, the AFP assay would have a prognostic value either for the evolution of liver regeneration or for the neoplastic transformation and would provide means to make the difference.

The program of work shall be to perform follow-up studies on the group of patients previously observed and to make repeated studies in all borderline cases. Such clinical studies will be correlated with liver radioisotope scintigraphy and liver needle biopsy for morphological information and with biochemical investigation of s-GOT, s-GPT, s-gamma-glutamyl-transpeptidase and the assay of HBsAg. All final reports will be elaborated with application of specially prepared computer programs.

29. MORPHOLOGICAL AND FUNCTIONAL DIFFERENTIATION OF GASTRIC CANCER WITH HIGH PLASMA ALPHA-FETOPROTEIN

Kameya, T., Kodama, T., Shimosato, Y., Okura, H., Kitaoka, H., Maruyama, K., Ministry of Health & Welfare, Pathology Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan*

OBJECTIVE: To study tumor markers of gastric cancers at the cellular level.

APPROACH: The project is carried out by immunohistochemistry of tumor tissues and by radioimmunoassay of patients' sera and tumor tissues on alpha-fetoprotein (alpha-FP), carcinoembryonic antigen (CEA), chorionic gonadotropin, and human serum proteins.

PROGRESS: Over 70% of the cases of gastric cancers contained CEA at cellular membranes and/or in cytoplasm. In over 20 cases of gastric cancer with high serum alpha-FP (400 ng/ml) histology showed features of poorly differentiated adenocarcinoma with medullary pattern, immunohistochemically positive cells possessing predominantly clear cytoplasm. Even in cases with curative surgery, only 13.2% survived 5 years, indicating grave prognosis of alpha-FP producing gastric cancer. Production of human serum proteins such as albumin, transferrin and anti-trypsin has been noted frequently in fetoprotein producing cases, as in liver cell carcinoma.

30. DETECTION OF EARLY PANCREATIC CANCER - ROLE OF RIBONUCLEASE, PANCREATIC ONCOFETAL ANTIGEN AND OTHER TUMOR-ASSOCIATED ANTIGENS (ABBREV)

Tytgat, G. N., Teunen, A., Huibregtse, K., de Vet, B., Hoek, F., Hoitsma, H. F., Universiteit van Amsterdam, Dept. of Medicine, *Spui 21, Amsterdam, Netherlands*

OBJECTIVE: Evaluation of various new methods for detection of pancreatic cancer (screening for pancreatic function abnormalities with PABA test and Lundh test; determination of ribonuclease, pancreatic oncofetal antigen, CEA and lactoferrin, in serum and in pure pancreatic juice; analysis of endoscopic retrograde pancreatographic findings in various diseases of the pancreas; correlation of the findings with the final state of patients with pancreatic cancer).

APPROACH: a) Determination of ribonuclease, pancreatic oncofetal antigen and other tumor-associated antigens in the serum of 100 patients with various abdominal malignant and nonmalignant conditions and of 100 normal age- and sex-matched control subjects. b) Detailed study of 100 patients, referred for a suspicion of possible pancreatic malignancy by 1) analysis of pancreatic functions using the PABA test, Lundh test, secretin pancreozymin test, fecal fat excretion rate, etc.; 2) determination of ribonuclease, pancreatic oncofetal antigen, CEA, and lactoferrin in serum and pure pancreatic juice; 3) critical analysis of radiological findings on the pancreatic ductal system obtained by endoscopic retrograde cholangiopancreatography; 4) cytological examination of pure pancreatic juice for exfoliating malignant cells; and 5) correlation of all the above findings with the final diagnosis and condition of the patients with and without pancreatic cancer.

31. IMMUNOCHEMICAL STUDIES OF GASTROINTESTINAL CANCER

Alpert, E. E., Massachusetts General Hospital, Medical Services, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

The summary for this project is under entry no. 22.

32. CONFORMATION OF THE ALPHA-FETOPROTEIN GENE IN CHROMATIN

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Previous studies have shown that micrococcal nuclease selectively excises genes which are in the transcriptionally active conformation in chromatin. The selectivity of this digestion process permits the fractionation of chromatin into transcriptionally active and inactive regions. In the proposed studies we will first use micrococcal nuclease as a probe for the alpha-fetoprotein gene in chromatin to determine if the loss of alpha-fetoprotein mRNA during normal liver development and the reappearance of alpha-fetoprotein mRNA during liver cancer are accompanied by corresponding alterations in the configuration of the alpha-fetoprotein gene in chromatin. Since chromosomal proteins are presumably responsible for the ordering of the DNA into template active regions, we will characterize these proteins associated with transcribed DNA in normal and malignant hepatocytes. In addition, chromatin reconstitution experiments will be employed to define the class of chromosomal proteins responsible for the generation of the alpha-fetoprotein genes into transcriptionally active conformations during normal and malignant development. These studies should therefore further our understanding of the transcriptional organization of chromatin during normal development and neoplastic transformation.

33. ONCO-DEVELOPMENTAL GENE CONTROL - ALPHA-FETOPROTEIN

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Possible mechanisms of control of gene expression for the onco-developmental serum protein alpha-fetoprotein (AFP) will be explored. Quantitation of the number of AFP genes in rat liver, kidney and Hepatoma 7777 indicates that AFP is transcribed from non-repetitive DNA and the number of AFP genes is approximately 2.0 for each of these tissues. Enhanced levels of AFP synthesis are observed in fetal and neonatal liver up to six weeks of age, after hepatocarcinogen exposure, following exposure to chemicals such as phenobarbitol, during restitutive proliferation after partial hepatectomy or chemically induced necrosis, and in hepatocellular and endodermal sinus (yolk sac) tumors. Data on neonatal liver and four selected transplantable hepatocellular carcinomas indicate that changes in AFP synthesis correlate with the cellular accumulation of AFP mRNA. These results suggest that the level of expression of AFP in these tissues is mainly regulated by modulating the steady-state concentration of the corresponding functional mRNA. The question as to how the steady-state level of AFP mRNA is modulated during liver development and oncogenesis remains to be answered. Although specific gene amplification cannot be ruled out completely, further investigation will concentrate on regulatory mechanisms involving transcriptional control or modulation at the level of RNA processing and/or transport into the cytoplasm. We intend to focus our attention therefore on the following: 1) Structure, organization and sequence of the AFP gene and possible relationships to transcriptional control. 3) Relative abundance, and complexity and stability of AFP messenger sequences in the nucleus and cytoplasm at steady state. 3) Identification of primary nuclear precursors, stability of nuclear AFP primary messenger transcripts, and pathways of processing to active mRNA.

These studies should not only provide significant insights into the relationship of AFP production to carcinogenesis but also provide basic information on gene control mechanisms of eukaryotic cells.

34. ALPHA-FETOPROTEIN - STRUCTURE AND FUNCTION

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A continuation of studies on different aspects of alpha-fetoprotein (AFP), the fetal protein associated with hepatomas and teratomas, is proposed. 1. We have recently developed a new purification procedure for bovine AFP. This method has allowed the purification of gram quantities of AFP. We now propose to carry forth the work on the amino acid sequence of AFP making use of this supply of bovine AFP. Our earlier studies have established amino acid sequence homology of AFP with albumin. 2. We have recently found that AFP binds bilirubin. This, and the structural similarity with albumin, suggests that other small molecules may show such binding. We propose to test AFP for binding of various small molecular weight ligands, especially ones that could influence cell differentiation and functions of immune cells. 3. We have recently noticed that human AFP has a subfraction which does not bind to concanavalin A. The amount of this subfraction is much higher in the AFP of teratocarcinoma patients. Some hepatoma sera also seem to have an elevated proportion of the nonbinding variant. These findings have opened new possibilities in the use of AFP in tumor diagnosis, which could allow a diagnostically useful distinction between cirrhosis and hepatoma if the AFPs produced in these conditions are different. We also propose to attempt to develop antisera specific for the subtypes using hybridomas. 4. We will prepare homogenous hybridoma antibodies to human AFP. Such antibodies, when well characterized and widely distributed, could remove one variable from the AFP assay. Some of them may be specific for the subtypes mentioned above. We also propose to utilize the hybridoma technology to produce large amounts of mouse antibodies to mouse AFP for use in immunotherapy experiments.

BIBLIOGRAPHIC REFERENCES: Ruoslahti, E., Estes, T., and Seppald, M., Binding of bilirubin and human alpha-fetoprotein. Biochim. Biophys. Acta 578: 511-519, 1979. Jalanko, H., and Ruoslahti, E., Differential expression of alpha-fetoprotein and gamma-glutamyltranspeptidase in chemical and spontaneous hepatocarcinogensis, Cancer Res. 39: 3495-3501, 1979.

35. CHARACTERIZATION OF FETAL ANTIGENS IN TUMORS

Coggin, J. H., Weppner, W. A., University of South Alabama, College of Medicine, Dept. of Microbiology & Immunology, *307 University Blvd., Mobile, Alabama*, 36688, United States of America

Embryonic antigens (EA) are shared by many species, including mice and humans. Tumors of all metazoans tested carry EA and EA expression seems to be a ubiquitous trait of the transformed cell. EA serves as an auto-antigen in syngeneic animal systems when present on developing embryos or on tumors and these antigens elicit a broad array of autochthonous immunological responses in the host which are poorly understood.

The work scope of this continuation proposal describes studies aimed at continuing the isolation, localization and characterization of embryonic antigens in chemically and virally induced tumors of rodents. The SV40 and 3 MCA sarcoma systems are used as the primary models. Fetal antigens have been isolated in soluble form from fetal cells and tumor cells and their immunogenicity correlated with antigen dose, timing, and assay system used to monitor tumor resistance induced by immunization with EA from fetal cells and/or tumor cells. New work proposed will focus on continued purification of these antigens. Studies aimed at distinguishing them from TSTA coded for by SV40 or induced by 3 MCA, and discovering the role of soluble EA in activating suppressor cell functions in potentiating tumor progression and the development of embryos and fetuses in pregnant rodents, are described.

BIBLIOGRAPHIC REFERENCES: Coggin, Joseph H., Jr. Comparison of SV40 Induced Mouse and Hamster Tumor Specific Transplantation Antigen. In Press, J. Natl. Cancer Inst. 1979. Adkison, L. R. and Coggin, J. H., Jr. In Vivo Function of Immune Murine Peritoneal Exudate Cells after Freezing and Thawing: Brief Communication. In Press, J. Natl. Cancer Inst. 1979.

36. STUDIES ON ALBUMIN AND ALPHA-FETOPROTEIN GENE EXPRESSIONS DURING HEPATOCARCINOGENESIS

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OBJECTIVE: Relationship between serum albumin gene expression and alpha-fetoprotein gene expression is studied during hepatocarcinogesis.

APPROACH: Serum albumin and alpha-fetoprotein levels are measured by immunological technique during hepatocarcinogensis in rats by 3'-methyl-DAB. Alpha-fetoprotein and albumin mRNA levels in the liver are assayed by RNA/cDNA hybridization. The effects of strain difference and modifying factors of hepatocarcinogenesis on mRNA and serum levels of these proteins are also studied.

37. REGULATION OF ALPHA-1-FETOPROTEIN GENE EXPRESSION

Belanger, L., Anderson, A., Dauphinais, C., Universite Laval, School of Medicine, Dept. of Biochemistry, *Ste. Foy, Quebec,* G1K 7P4, Canada

OBJECTIVE: To elucidate the mechanisms which control the expression of alpha 1-fetoprotein (AFP) during liver development and malignant transformation.

APPROACH: An AFP gene regulation analysis system is developed in the rat. A cDNA AFP probe serves in RNA/DNA hybridization assays to determine if the differential expression of the AFP gene during particular physiological states of the liver involves a change in chromatin template specificity. Our approaches to the identification of nuclear elements regulating the AFP gene sequence are through non-histone protein characterization and nuclear and chromatin transcription and reconstitutions in vitro. Particular attention is devoted to the sequence of molecular events by which glucocorticoid hormones reprogram the protein synthesis scheme in perinatal liver, resulting in the premature extinction of the AFP function (Belanger et al., Nature 256: 657, 1975).

PROGRESS: We have purified and characterized mRNA AFP and synthesized a cDNA probe (Cancer Research 39: 2141, 1979): Translation and hybridization data with polysomal, total cytoplasmic and total cellular RNA indicate that the suppression of AFP by dexamethasone in newborn rat liver is highly selective and operates at the transcriptional level of gene regulation (submitted for publication).

38. ALPHA-FETOPROTEIN REGULATION IN FETAL AND CANCER LIVER

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Our overall objective is to elucidate the mechanisms that regulate gene expression at the level of transcription in mammalian cell, and, in particular, the mechanisms that regulate alpha-fetoprotein (AFP) gene transcription during ontogenesis and hepatocarcinogenesis. We attempt to determine if the differentiation and hepatocarcinogenesis involves a change in the transcriptional activity in the cells, and to identify the nuclear macromolecules that affect AFP gene transcription. In this project, we also study the effects of hormones on AFP synthesis in developing rat liver with the purpose of creating a new approach to the study of AFP regulation and its relationship to liver cell differentiation and malignant transformation.

BIBLIOGRAPHIC REFERENCES: Belanger, L., Chiu, J-F., Exton, J.H., Richardson, D., Laberge, C., Larochelle, J., and Belanger, M., Regulation between adenine nucleotide and AFP metabolisms in liver injuries, in Carcinoembryonic Proteins: vol II (Lehmann, F.G. ed.) Elsevier, North Holland, pp. 225-229, 1979. Belanger, L., Commer, P., and Chiu, J-F., Isolation of rat alpha-1-fetoprotein messenger RNA. Cancer Res. 39, 2141, 1979.

39. REGULATION OF ALPHA-FETOPROTEIN GENE EXPRESSION

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The long-term goal of the research is to gain a thorough understanding of the factors which regulate the expression of alpha-fetoprotein (AFP) in normal and transformed cells. During development, AFP is synthesized in the yolk sac and liver of the mouse embryo. After birth, the amount of AFP in serum decreases markedly to a basal level which represents less than 0.01% of that in fetal serum. However, reinitiation of AFP synthesis in the adult occurs in liver following injury, in hepatocarcinoma cells, and testicular teratocarcinomas. In addition, a number of congenital birth defects, including congenital nephrosis, severe Rh-hemolytic disease and several open neural tube defects are associated with increased concentrations of AFP in the maternal and/or fetal circulation and amniotic fluid. In view of the diagnostic value of elevated serum AFP levels in detecting these pathological states in humans, it is clearly important to understand at a molecular level the factors responsible for this elevation. Therefore, I intend to isolate and clone that segment of the mouse genome containing AFP transcribed and flanking sequences which are involved in its regulated expression. The detection of the AFP genomic fragment in the bacteriophage lambda gt WES lambda B vector will be performed using a chimeric plasmid containing a portion of the AFP mRNA sequence which has been constructed. The genomic segment will be used for extensive sequence analysis of the structure and organization of the AFP transcription unit, and as a substrate for in vitro transcription assays designed to identify the role of RNA, histones, and non-histone chromosomal proteins in the expression of AFP.

BIBLIOGRAPHIC REFERENCES: Gorin, M.B. and Tilghman, S.M. The structure of the alpha-fetoprotein gene in the mouse. Proc. Natl. Acad. Sci., U.S.A., in press, 1980.

40. HUMAN TUMOR ANTIGENS - ANTIGENIC ANALYSIS OF HUMAN FETAL SPLEEN

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The objectives of these investigations are to determine the existence of fetal-specific human splenic antigens, to characterize these materials physiochemically and immunochemically and to determine if assays for these constituents have any relevance to human disease.

Rabbits were immunized with aqueous extracts of human spleen and the antisera raised were then tested with a variety of both fetal and adult human tissues. The studies were carried out both by the use of precipitin-analysis in agar gel and by immunofluorescence studies. The physiochemical characterization of the material has been initiated by gel sieve chromatography and polyacrylamide gel electrophoresis.

To date, four human fetal splenic antigen systems have been demonstrated. As expected, alpha1-fetoprotein was detected, and this system put aside. Interesting observations have been made concerning fetal hemoglobin (HbF) and a radioimmunoassay for the gamma chain of HbF has been established and is presently being examined in a variety of human disease states. In addition, immunofluorescence studies have revealed at least one T lymphocyte and one lymphocyte marker which have not, to the best of our knowledge, been described to date.

41. THE BIOLOGICAL ROLE OF ALPHA-FOETOPROTEIN

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OBJECTIVE: The biological parameters which accompany the appearance of alpha-foetoprotein in serum have been studied by many authors; the production of alpha-foetoprotein by transitional cells is related to cellular proliferation. The study of the role of this protein in tumorigenesis is the main aim of this project.

APPROACH: The role of AFP is being studied using models at the cellular level; cell fusion using PEG will be used to elucidate whether the production of AFP by neoplastic cells can be inhibited by fusion with normal hepatocytes. The production of AFP will be compared with other neoplastic properties. The hypothesis that AFP is a carrier of a growth factor will be tested using the hepatocyte cell culture and by binding studies in vitro.

The relationship between the appearance of AFP and another marker of hepatoma, gamma-glutamyl transpeptidase, as well as the phenotype expression of GGT in neoplasia, will be studied.

PROGRESS: Quantitative modifications of GGT were observed during chemical carcinogenesis but no GGT isoenzyme difference could be observed after tumor induction with 3'-Me-DAB as indicated by ConA-Sepharose chromatography, electrophoresis and Km determination. GGT elevation in our conditions seems to be related to a detoxication process rather than to derepression.

42. THE TURNOVER OF ALBUMIN AND ALPHA-FETOPROTEIN MRNA'S DURING EMBRYONAL DEVELOPMENT AND ONCOGENESIS IN RAT LIVER

Yap, S. H., Selten, G. C., University Hospital of Nijmegen, Dept. of Medicine, *Geert Grooteplein 10, 6525 GA Nijmegen, Netherlands*

OBJECTIVE: Albumin is a specific protein synthesized in the liver. The synthesis of albumin is initiated before birth but is completely activated after birth. In hepatoma cells, the albumin synthesis can be reduced or abolished. The lack of albumin production in certain hepatoma cell lines has been ascribed to a general defect in the processing and secretion of liver-specific proteins; however, in other cell lines with defective albumin synthesis, no albumin mRNA is present. Many neoplastic cells resemble more primitive cells and, like embryonic cells are deficient in adult functions. Furthermore, the synthesis of antigens of the fetal type by malignant cells has been observed in many cases. Alpha-fetoprotein, which is synthesized by the parenchymal cells of the embryonic liver, exists only in trace amounts in normal adults. However, in most adults bearing hepatomas, the production of alpha-fetoprotein is dramatically enhanced. By studying the turnover of albumin and alpha-fetoprotein mRNAs during fetal and tumorous development of rat liver, the 'mechanism' of 'turn-off' and 'turn-on' of gene expressions will be studied.

APPROACH: Albumin and alpha-fetoprotein mRNAs will be quantitated by the method of molecular hybridization using specific albumin and alpha-fetoprotein DNA complementary to mRNA's.

The synthesis or production of albumin and of alpha-fetoprotein will be examined in isolated cells as well as in cell-free protein synthesis.

43. ALPHA-FETOPROTEIN SYNTHESIS BY HEPATOMA CELLS IN VITRO

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The purpose of this investigation is to study an oncodevelopmental protein produced by tumor cells growing in cell culture. The synthesis, metabolism, molecular variants and physicochemical properties of rat alpha-fetoprotein (AFP) produced by mass and clonal cultures of cells derived from hepatocellular carcinomas will be studied. AFP produced by the cells in culture will be compared to AFP produced in vivo by normal fetal/newborn animals and by the hepatocellular carcinomas. Hepatocellular carcinoma-derived cells are grown using methods which have been modified to support the growth of epithelial cells. Mass and clonal cell lines have been established from Morris hepatoma 7777 and do produce AFP. The relationship of these cells to liver parenchymal cells will be established by cell morphology, albumin synthesis, and tyrosine aminotransferase activity. The synthesis, processing, excretion and molecular variants of AFP produced by the cultured cells will be characterized. Varying culture conditions, hormones, cyclic nucleotides and carcinogens on AFP production in vitro will be tested. Chromosome analysis of the cells will be performed using conventional and banding methods. AFP will be isolated by anti-rat AFP immunoadsorbent affinity chromatography. Concanavalin A-affinity molecular variants of AFP will be isolated by concanavalin A-affinity chromatography. Electrophoretic molecular variants of AFP will be isolated by preparative isoelectric focusing. The amino acids composition, peptide content, terminal amino acids, and carbohydrate content of the molecular variants of AFP produced by cells in culture will be determined by standard methods. Molecular size of AFP's will be determined by SDS-acrylamide gel electrophoresis and by gel filtration chromatography.

BIBLIOGRAPHIC REFERENCES: Kelleher, P.C. 1979. Urinary excretion of hydroxyproline, hydroxylysine and hydroxylysine glycosides by patients with Paget's disease of bone and carcinoma with metastases in bone. Clin. Chim. Acta. Smith, C.J., P.C. Kelleher, L. Belanger and L. Dallaire. 1979. Reactivity of amniotic fluid alpha-fetoprotein with concanavalin-A in diagnosis of neural tube defects. Brit. Med. J. in press.

44. HEPATOMA ALPHA-FETOPROTEIN - CHEMISTRY AND METABOLISM

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Four molecular variants of rat alpha-fetoprotein (AFP) have been identified based on their concanavalin A affinity (ConA-A) and isoelectric points. The ConA-A molecular variants present in amniotic fluid, and in sera of fetal/newborn rats, primary and transplantable hepatoma-bearing rats and rats during 3'-MDAB hepatocarcinogenesis have been measured previously. Each of the electrophoretic molecular variants will be quantified in amniotic fluid, fetal and newborn rat serum, serum of rats undergoing 3'-MDAB hepatocarcinogenesis, serum of rats bearing 3'-MDAB-induced primary hepatomas, serum of rats bearing transplantable hepatomas derived from the 3'-MDAB-induced tumors, serum of subtotally-hepatectomized rats and serum of rats exposed to hepatotoxins (CC14, galactosamine). ConA-A molecular variants will be measured in sera of adult rats, of rats previously exposed to hepatotoxins and of rats which have undergone subtotal hepatectomy. The electrophoretic variants present in each of the ConA-A molecular variants of the AFP in amniotic fluid and in sera obtained from fetal rats, new-born rats and hepatoma-bearing rats will be measured. Each of the four known molecular variants will be isolated from sera of newborn rats, primary hepatoma-bearing and transplantable hepatoma-bearing rats. The purified variants will be characterized and compared physicochemically and immunologically. The metabolisms of the ConA-A and electrophoretic AFP molecular variants will be compared in normal adult rats. Half-lives of the molecular variants will be calculated, and the possibility of interconversion among the molecular variants will be tested. The objectives of this investigation are to 1) establish the pattern of AFP molecular variants present under different physiologic and pathologic conditions, 2) determine if there are differences in the metabolism of the molecular variants following secretion from the liver cell, and 3) determine the physicochemical differences among the various molecular forms of rat AFP.

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45. ONCO-DEVELOPMENTAL (CARCINOEMBRYONIC) PROTEINS

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OBJECTIVE: Biology and medicine of alpha-fetoprotein (AFP).

APPROACH: Study on metabolism and molecular biology of AFP.

Study on anti-tumor effect of anti-AFP serum. Differentiation of tumor cells into normal cells.

PROGRESS: Purification of mRNA of AFP is accomplished. cDNA is in preparation. Bioengineering of AFP will be studied. Antihepatoma and testicular tumor cell effect of anti-AFP serum was demonstrated experimentally. Clinical use of the antiserum is progressing. Rat yolk sac tumor cells were shown to differentiate into normal cells in vitro. Conditions necessary for differentiation are under investigation.

46. EMBRYONIC AND VIRALLY INDUCED TUMOR-CELL MEMBRANE ANTIGENS

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Expression of chicken fetal antigens (CFA), thirteen serologically distinct oncodevelopmental membrane antigens, will be analyzed in embryonic development and in cellular differentiation of erythroid and myeloid cells in normal and cancerous chickens, as well as in in vitro cell lines. The possibility that CFA is an important membrane recepior for cell-cell communication in embryonic development will be investigated by analyzing CFA expression on the primitive erythroid series in normal 12 hour-11 day embryos and screening for CFA abnormalities in previously identified developmental mutants. The possibility that CFA determinant expression may be associated with specific cellular differentiation-cell cycle phases will be analyzed in normal and erythroleukemic chickens, and virally transformed lymphoid cell lines. Breeding experiments will be performed in order to determine the inheritance pattern of CFA determinants. The role of genetic versus environmental factors in CFA expression will be investigated in in vitro analyses of erythroid cellular maturation of cells obtained from normal embryos and adult bone marrow. The possibility that CFA may serve as a viral receptor will be further analyzed. The relationship of AFP and suppressor T lymphocytes, both expressed during embryonic development, to suppression of the immune response system and tumor induction will be investigated.

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47. ALPHA-FETOGLOBULIN (HUMAN, RATS)

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This is part of a broader project. A summary of this subproject is not available.

48. IMMUNOCHEMICAL STUDIES OF ALPHA1-FETOPROTEIN (AFP) ASSOCIATED WITH HUMAN AND OTHER MAMMALIAN HEPATOCELLULAR MALIGNANCIES

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OBJECTIVE: To determine if human alpha1-fetoprotein (AFP) manifests any immunosuppresive properties based on I region restrictions.

APPROACH: Investigations will be undertaken in which purified cord blood AFP will be examined for its differential binding to autologous fetal cord lymphocytes, maternal lymphocytes, paternal lymphocytes and those of unrelated individuals in the population. The assay will involve the binding of the radiolabelled purified AFP to the lymphocytes in question and, in a parallel fashion, the use of affinity purified monospecific 125I-anti-AFP to the same populations of lymphocytes. Should differential binding be established in the fetus and/or mother and/or father, then the specific areas of the molecule involved in the binding phenomenon will be sought and compared with the immunogenic fragements of AFP.

PROGRESS: Recent studies in our laboratory have led to the development of a method for the physicochemical isolation of purified AFP (Cancer Research, Vol. 38, 6-12, Jan. 1978). These preparations have failed to demonstrate any immunosuppresive activity, as had previously been suggested (J. Immunol. 119: 897, 1977). More recently, the suggestion has been made that the immunosuppressive function of the AFP molecule is restricted in a fashion related to I region determinants within the major histocompatibility complex. It is this hypothesis that will be examined.

49. HUMAN TUMOR ANTIGENS - STUDIES IN HUMAN ALPHA1-FETOPROTEIN

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The major objective of the work and progress is to assess the immunoregulatory properties of human alpha1-fetoprotein (AFP) both in allogeneic and xenogeneic systems.

Human AFP will be purified by a number of different techniques. These will include the physicochemical approach to the purification of human AFP previously described in this laboratory, as well as a number of other techniques involving immunoprecipitation and affinity chromatography. It may well be that different AFP populations will be obtained by these different procedures so that some information may be forthcoming as to what specific subpopulation of AFP molecules, if any, is involved in immunoregulation. The systems of immunoregulation to be studied will include those involving MLR as well as lectin-stimulated lymphocytes. An attempt will be made to assess the involvement of the analogue of I-region triggering in the mouse vis-a-vis AFP immunoregulation. A number of studies will be undertaken aimed at determining a structure-function relationship in the human AFP molecule.

In the studies completed, a RIA for AFP and its clinical utility have been described and a physicochemical technique for the purification of human AFP has been established. Employing ascites fluid as the starting source of material, 'monoclonal' human AFP has been obtained. Initial studies of this material indicate that human AFP preparations do not have universal immunoregulatory effects so that any such property would have to be attributable to either a subpopulation of molecules or to a contaminant of AFP preparations employed in other investigations. Further studies along these lines are in progress.

A variety of studies involving the assay of AFP in the circulation of pregnant women have led us to caution against the indiscriminant use of circulating maternal AFP as an indication for abortion during the later part of pregnancy or as a method for 'ruling out' neural tube defects (NTD) at any time during pregnancy. The possibility of such an assay as an adjunct in the diagnosis of NTD still remains a possibility.

50. SEARCHING FOR ALPHA-FETOPROTEIN AG-AB COMPLEXES IN PLACENTAS

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The purpose of this study is to see if antibody (Ab) to AFP can be identified in human placentas including those from patients with toxemia. So far we have tentative evidence that such complexes are present in, at least, one placenta from a patient with toxemia. AFP seems to be an immunosuppressive compound and the human host appears to be tolerant of its presence during both gestation and malignancies such as carcinoma of the liver. Whether it is responsible for human tolerance to the placenta as well as to certain malignancies is not known. However, perturbations in the host response to this compound may be relevant not only to normal pregnancies but to certain tumors as well.

51. THE IMMUNOBIOLOGY OF ALPHA-FETOPROTEIN IN ONCOGENESIS

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It has been well documented that embryonic or fetal antigens can be identified in the sera of mice and humans with a variety of neoplasia. Although the study of fetal antigens has largely emphasized their identification for the clinical diagnosis of malignancy, recent information suggests that such fetal antigens may have direct influence on both general host immunocompetence and specific tumor immunity. In particular, it has been demonstrated, using alpha-fetoprotein (AFP) purified from mouse amniotic fluid, that under specific experimental situations AFP may function as a suppressive agent for immune responsiveness. Unfortunately, the specificity of this suppression, its general role as an immune regulator, its association with other materials, and its possible function as a tumor promoting agent, are sources of controversy. In our laboratory, over the past year using select models of tumor induction, we have documented a broad, probably antigen non-specific, but perhaps organ specific, interaction of AFP at several levels. For example, treatment of mice with AFP appears to stimulate the production of a suppressor T cell population which markedly alters the natural history of Moloney sarcoma virus induced tumors. Furthermore, AFP administration influences both spontaneous leukemia in AKR/J mice and autoimmunity in New Zealand black mice. Similarly, in genetically susceptible mice (B6C3), AFP appears to be a co-promoter of diethylnitrosamine and dieldrin induced hepatomas. In contrast, AFP appears not to influence the induction of methylcholanthrene or DMBA tumors. Because these interactions of AFP appear to be multi-factorial, it is planned to further identify and define these select features, extend them to other models of oncogenesis, and in particular, to address organ and lymphocyte subpopulation specificity. These will be accomplished by: 1) Defining the influence of AFP on ontogenic influences of the immune response; 2) characterization, including Ly phenotyping, of the suppressor cell population generated by AFP; 3) identification of the mechanism of AFP acceleration of AKR/J leukemia; 4) study of the influence of AFP on reversibility of hepatic nodules; and 5) defining the additional mechanisms of AFP modulation of host responsiveness.

52. REGULATION OF THE IMMUNE RESPONSE IN ADULT ANIMALS TO ANTIGENS FIRST ENCOUNTERED IN THE NEONATE

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OBJECTIVE: To investigate the effect of neonatal response to antigen on the response of adult animals on re-exposure to those antigens.

APPROACH: In order to understand these events we are currently investigating two different systems. In the first, we are studying the development of cytotoxic killer T cells into semiallogeneic cells in mice inoculated with those semiallogeneic cells at birth.

Tolerance in this case is to H-2 incompatible cells. In the second system we are analyzing the response of adult mice to antigens expressed on syngeneic embryo fibroblasts.

PROGRESS: Tolerance to MHC incompatible cells is maintained by suppressor cells. Two types of suppressor cells are detectable. The first acts to inhibit the induction of killer cells (from their immediate precursors) in culture. The second seems to be able to inhibit the production of cytotoxic cell precursors from (multipotential?) stem cells in vivo.

53. USE OF MANIPULATION OF IMMUNE RESPONSES TO EMBRYO-ASSOCIATED ANTIGENS IN THE IMMUNOTHERAPY OF LOCAL AND WIDESPREAD MALIGNANT DISEASE IN MICE

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OBJECTIVE: We are interested in exploring further the role of embryo-associated antigens in the immune response to spontaneously appearing murine adenocarcinomas.

APPROACH: To date our evidence points to developmental-stage unique embryonic antigens as playing a role in immune sensitization or the regulation thereof, in an experimental system in which we measure subcutaneous growth of these tumors.

As a way of examining the embyronic antigens themselves in more detail we intend to immunize C3H/HeJ mice with crude embryo antigen preparations, and prepare monoclonal antibodies (from the spleen of these animals) by fusion with the tumor line P3X63Ag8. These antibodies will be used not merely to characterize the antigens of the tumor cell itself, but also to manipulate the response within the host of origin of that tumor. Before attempting the latter, however, it will be necessary to know more about the cellular interactions involved in regulating the response to embryo-associated antigenic determinants, and how these interactions can be manipulated in other model systems. To this end we are continuing to investigate idiotype-anti-idiotype regulation of antibody responses to soluble protein antigens, and of cytotoxic responses to alloantigens. In addition, we are analyzing the role of the host 'thymic environment' in controlling the receptor repertoire for responses to xeno-, allo-, and syngeneic (especially embryo-associated) antigenic determinants.

54. TERATOCARCINOMA AND EMBRYONAL TUMORS - SURFACE ANTIGENS

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This application proposes to follow serologically embryonic surface antigens in the mouse that are characteristic of specific early stages of cell differentiation both in the embryo and on embryonal tumor cells, specifically teratocarcinoma and a genetically caused medulloblastoma-neuroblastoma. The project will make use of a series of mutant genes at the T-locus which are known to: 1) Interfere with differentiation at specific times in development, 2) specify cell surface antigens on sperm and in the embryo, and 3) in the one case studied, cause an embryonal tumor. We would like to know the time course of the expression of these antigens, if they are functional, and what role the mutant form of the antigen plays in malignant differentiation. These objectives involve transplantation of mutant embryos, obtaining transplantable tumors from t-mutant cells, and serological definition of embryonic surface antigens by means of cytotoxicity tests and absorption studies.

55. ONCOFETAL ANTIGENS IN MOUSE EMBRYOGENESIS AND TUMOR GROWTH

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The objectives of this research are to detect and characterize embryonic and oncofetal antigens of various embryonic systems and a tumor system, and to assess the involvement of these antigens in pre-implantation mouse embryogenesis and tumor growth. Immunological techniques for antigen recognition and functions are being employed and include: (1) Mixed lymphocyte reactions, (2) cell-mediated lympholysis, (3) lymphocyte-induced growth inhibition, (4) antibody-induced growth inhibition, (5) cross-reactivity of serologically defined and lymphocyte defined antigens, and (6) scanning electron microscope localization of defined antigens on preimplantation mouse embryos. These experiments should further our understanding of the role of oncofetal antigens in preimplantation mouse development and tumor growth.

56. IMMUNOBIOLOGICAL PROPERTIES OF EMBRYONIC ANTIGENS

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The physical-chemical properties of tumor-associated fetal antigens (TAFA) have been carefully delineated in order to better understand their biological activities. TAFA are closely associated with rat histocompatibility (H) antigens on transformed cell membranes. These are noncovalent interactions; TAFA and H antigens do not coprecipitate in immunoprecipitation reactions; and when these two different classes of antigens are solubilized from tumor or fetal cells, it was shown that TAFA are not cleavage products or fragments of larger macromolecules.

It was important to characterize these properties of TAFA because they produce significant suppression of mitogen, syngeneic tumor cells, as well as alloantigen induced lymphocyte proliferation. The immunosuppression is reversible and nanogram quantities are effective in elicitation of suppression.

Current efforts are to determine the mechanism(s) of induction of suppression. These studies are currently being conducted and involve: determination of lymphocyte binding by different TAFA (saturation kinetics, specific vs. nonspecific binding by primed lymphocytes, etc.); and efforts to find the target cell of the TAFA induced suppressive effects are in progress.

57. HEPATOCELLULAR CARCINOMA IN URBAN BLACKS - ETIOLOGICAL FACTORS AND RELATIONSHIP TO CERTAIN ONCO-FETAL ANTIGENS

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Hepatocellular carcinoma has a high incidence in Southern Africa. Numerous series have shown a strong relationship with HBV. A preliminary study was performed using orcein staining for HBsAg on autopsy material obtained from patients dying of hepatocellular carcinoma at Baragwanath Hospital. A low positivity was noted. This was extended in a study using immunoperoxidase and aldehyde-fuchsin staining techniques on similar material. Seventeen percent of the Baragwanath series showed HBsAg positively in non-neoplastic liver as opposed to 72% in a control group of Shangaan miners from Mozambique. A significant number of cases showed iron overload and micro-nodular cirrhosis in the Baragwanath series. A prospective study is now commencing using serological and immunochemical techniques to investigate the relationship between various tumor-related antigens, including HBsAg, alpha-fetoprotein, carcinoembryonic antigens and ferritin, and hepatocellular carcinoma in the urban black population.

58. MULTIDISCIPLINARY CANCER DIAGNOSIS AND THERAPY

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A new oncofetal antigen (OFA-1) has been identified on biopsied and cultured human tumors of diverse histologic origin including over 50% of 170 biopsied melanomas, sarcomas, and carcinomas. OFA-1 was not detected on any of 61 normal adult tissues, but was detected on fetal brain tissues by serologic absorption. OFA-1 was induced on normal fibroblasts after in vitro culture. OFA-1 is immunogenic in man, unlike other fetal antigens (CEA and AFP), since antibody to OFA-1 is found in sera of many cancer patients and is induced in cancer patients immunized with OFA-1 positive allogeneic tumor cells.

Tumor associated antigens were isolated from melanoma cells cultured in a serum-free chemically defined medium. The purified antigens were cross-reactive with other melanomas, but not with sarcomas or fetal tissues. The purified antigen was labeled with 125I and used to develop a radioimmunoassay with melanoma patients' sera. The assay will be tested for sensitivity in detecting soluble antigen in sera from advanced cancer patients and a similar assay will be developed with the oncofetal antigen. We will continue to investigate antigenic modulation and other immune mechanisms that account for survival of antigenic tumor cells in patients with high levels of antibody.

59. TUMOR MARKERS IN NEOPLASIA

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PROJECT OBJECTIVES: 1) The ectopic production of proteins with potent biologic effects. For example, hormones or embryonic antigens provide markers for certain malignant tumors. The cancer cell therefore may exhibit dedifferentiation to a more primitive cell type with activation of genetic loci that are normally repressed. 2) The discovery of simple chemical compounds that activate loci for the production of the fetal hormone, human chorionic gonadotropin (HCG), provides a system for analyzing gene activation as observed in tumors. 3) Activation of these genetic loci might be used to detect occult malignancies.

PLAN: To isolate mutant tumor cell strains resistant to the growth inhibitory effects of compounds that activate dormant genetic loci. These mutant cells might permit a genetic dissection of the induction process.

METHOD: Clone cell lines resistant to the growth inhibitory effect of mediators of hormone induction. Determine by cell fusion methods the dominance of mutation. Analyze molecular basis of effect.

CLINICAL RELATIONSHIPS: This area of research may provide new methods for detecting occult neoplasms or determining the presence of metastasis.

60. HUMAN BLOOD AND TISSUE PROTEINS

Deutsch, H. F., Nagai, M., Schmolke, B., Jabusch, J. R., Evenson, M., Hanson, K., Becker, J., University of Wisconsin, Madison Campus, Medical School, Dept. of Physiological Chemistry, *307 N. Charles St., Madison, Wisconsin*, 53706, United States of America

Alpha-Fetoprotein: The effect of both human and rat sources of this protein and their complexes with specific fatty acids will be individually investigated as to their effects on mitogen induced lymphocyte transformations, (human and rat), antibody formation (rat) and tissue graft retention (rat). Studies will be continued on the structure of human source protein to define a model of the molecule and its relation to serum albumin.

Metalloproteins: Some preparations of bovine superoxide dismutase have extremely low E265-655 nM absorption ratios. The relatively high absorption at 655 nM suggests the presence of a prosthetic group or a labile structure which absorbs at this wavelength. Attempts will be made to resolve this question.

The human fetal extracts used to prepare alpha-fetoprotein will be used to generate metallothionine in studies designed to explain the shifts with age of this protein being complexed initially only with copper to one predominantly carrying cadmium. The relationships of metallothionine to chelatin will be investigated.

Immunoglobulins: Studies of the serological properties of human lambda-chains as they relate to primary structural variations will be continued. Attempts to correlate these properties with any tendency to amyloid formation will be made.

61. ANALYSIS OF RELATIONSHIP BETWEEN POLYOMA-INDUCED SURFACE ANTIGEN AND ONCOFETAL ANTIGENS AND H-2 ANTIGENS

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OBJECTIVE: At least three types of surface antigen have been described on the surface of polyoma transformed cells: a) TSTA, revealed by homograft rejection; b) TSSA, identified by antibody-mediated reaction; c) oncofetal antigens, located on mouse embryo cells. We propose to define relationships among these various antigens.

APPROACH: Using solubilization and purification techniques, we have partially separated these three types of antigens. By further purification and use of lymphocytotoxicity and chromium release, we will identify relationships among these various antigens.

PROGRESS: We have been able to demonstrate that TSSA comprises an oncofetal antigen and TSTA. These two antigens are different. H-2 antigen is not related to TSSA or TSTA. The purification of these various antigens is in progress.

62. FETAL ANTIGEN SURVEY (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

II. ANTIGENS OF CHEMICALLY INDUCED TUMORS

63. ISOLATION AND CHARACTERIZATION OF TUMOR-SPECIFIC ANTIGENS OF EXPERIMENTAL ANIMAL TUMORS AND HUMAN CANCER

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OBJECTIVE: Isolation of chemically-induced tumor-specific antigens (TSA) of rat tumors and the isolation of human TSAs.

APPROACH: Papain-digestion of tumor-cell membranes and the use of affinity chromatography with syngeneic antiserum in the animal tumor model and other types of antisera in the human tumor systems.

PROGRESS: Isolation of animal and human TSAs suggests that they are linked to B2m.

64. CHARACTERIZATION OF TRANSPLANTATION ANTIGENS OF 3-METHYLCHOLANTHRENE-INDUCED MURINE SARCOMA (ABBREV)

Parmiani, G., Carbone, G., Invernizzi, G., Sensi, M. L., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To purify, isolate and biochemically characterize the tumor-associated transplantation antigens (TATA).

APPROACH: In vivo passaged methylcholanthrene induced murine fibrosarcomas will be used throughout the experiments. Microsomal fractions defined as crude membranes (CM) will be obtained from tumor fragments passed through a mechanical press and subsequent differential centrifugations. A crude soluble (CS) preparation prepared after detergent solubilization of CM, will be fractionated by gel filtration (Sephadex G200) and then submitted to affinity chromatography on lectin columns. CM, CS and the subsequent fractions will be tested for in vivo TATA activity. Attempts to raise TATA antisera will be made by injection of purified material endowed with TATA activity into syngeneic or allogeneic mice. The availability of TATA antisera would allow further purification of TATA molecule by immunochemical methods and subsequent analysis of precipitates by SDS-PAGE.

65. RELATIONSHIPS BETWEEN HISTOCOMPATIBILITY ANTIGENS AND TUMOR SPECIFIC TRANSPLANTATION ANTIGENS OF METHYLCHOLANTHRENE-INDUCED SARCOMAS

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OBJECTIVE: To isolate, biochemically characterize and compare the tumor specific transplantation antigens (TSTA) with the histocompatibility antigens of methylcholanthrene-induced murine fibrosarcomas.

APPROACH: Fibrosarcomas are induced in BALB/c, B10, B10.BR, B10.D2 and B10.M mice by subcutaneous application of methylcholanthrene. After 3-4 in vivo passages the tumors are then examined for the presence of TSTA by in vivo transplantation techniques, and for the expression of normal and abnormal MHC products by serological and biochemical methods. A panel of syngeneic and semisyngeneic anti-TSTA sera is being raised in appropriate hosts to produce reagents to be used in RIA against soluble, partially purified TSTA.

66. ANTIGENS OF CHEMICALLY TRANSFORMED MOUSE FIBROBLASTS

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I propose to identify cell surface antigens that are present on chemically transformed mouse fibroblasts but not on normal fibroblasts. My strategy will be to fuse myeloma cells with spleen cells from rats immunized with fibroblasts transformed in vitro with methylcholanthrene, so as to produce antibody-secreting somatic cell hybrids (hybridomas). Hybridomas producing monoclonal antibodies that bind to the transformed fibroblasts used for immunization, but not to normal fibroblasts, will be identified by an autoradiographic indirect 125I-protein A binding assay and cloned. The monoclonal antibodies will be purified either from spent culture medium or from the ascites fluid of nude mice or rats bearing hybridomas. The purified antibodies will be labelled with 125I and used to test a number of transformed and revertant fibroblast lines. Antibodies that identify transformation-specific antigens will be tested on a more extensive panel of cell types. The antigens identified will be purified from radiolabelled cells by immunoadsorption on antibody-Sepharose columns and sodium dodecyl sulfate-polyacrylamide gel electrophoresis for subsequent structural analysis, which will comprise molecular weight determination, peptide mapping, and partial amino acid sequence determination.

67. CELL SURFACE ANTIGENS OF CHEMICALLY INDUCED SARCOMAS

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We shall continue work, as outlined in our original application, trying to define various tumor-associated antigens in chemically induced mouse sarcomas, including tumor specific transplantation antigens that are essentially unique for each sarcoma, possibly occurring sarcoma-specific antigens shared by most or all sarcomas, as well as various differentiation antigens. The involvement of antigens of MuLV will, as in the past, be investigated as well. Serological methods will be used primarily, coupled with transplantation assays as needed. The Milstein-Kohler hybridoma technique will be the method of choice for most of our experiments.

BIBLIOGRAPHIC REFERENCES: Hellstrom, I., Hellstrom, K.E., and Bernstein, I.D. Tumor-enhancing activator T cells in spleens and thymuses of tumor immune mice. Proc. Natl. Acad. Sci. USA 76:5294-5298, 1979.

68. SOLUBILIZATION AND PURIFICATION OF TUMOR-SPECIFIC TRANSPLANTATION ANTIGENS

Kahan, B. D., University of Texas, Health Science Center at Houston, School of Medicine, Dept. of Surgery, *Box 20036, Houston, Texas,* 77025, United States of America

Tumor extracts offer the opportunity to dissect distinctive characteristics of neoplastic cells. Immunization of syngeneic hosts with materials released by 3M KCl from the surfaces of murine methylcholanthrene-induced sarcomas evokes specific resistance toward challenge with 100 times the lethal dose of tumor, demonstrating the presence of tumor-specific transplantation antigens (TSTA). The applicant and his colleagues have subjected 3M KCl extracts of C3H/HeJ tumors MCA-D, MCA-F, and MCA-T to biochemical and immunological analysis. Materials capable of evoking specific immunoprotection at 5 mcg doses were obtained from several tumors by preparative isoelectric focusing to obtain Fr 15 (pIEF; pI 5.8). Resistance induced by the pIEF fraction was transferred to normal, syngeneic recipients by radiosensitive, non-adherent, thymus-dependent lymphocytes. The antigen behaved like a weak immunogen with a narrow dose response range, and did not contain gp 70 determinants in competition assays with standard Gross lymphoma and Friend-Moloney-Rauscher reagents. Tumor-specific-antisera raised by multiple cycles of neoplastic challenge and resection were specifically inhibited by Fr 15, thus providing excellent reagents for the dissection of the antigenic determinant(s) eliciting host immune responses. While the proposed experiments will dissect the issue in greater detail, preliminary transplantation experiments did not demonstrate H-2 histocompatibility determinants in Fr 15. In addition to the immunoprotective principle, pIEF demonstrated the presence of an antagonistic material (Fr 1, pI 2.5) evoking immunologically specific tumor growth facilitation, which was transferred to normal hosts by Thy 1.2 sensitive, radioresistant lymphocytes. Multiple injections of Fr 15 after challenge with a large neoplastic burden (10 to the 6th power cells) overcame the normal sensitivity of hosts who had rejected modest tumor loads (10 to the 5th power cells). The proposed work will examine the immunotherapeutic effect of Fr 15 both in primary hosts and in animals treated by incomplete resection with appreciable residual tumor burden. Further, the proposed studies will purify the active principles in Fr 1 and 15. (Text Abridged).

69. ANTIGENIC HETEROGENEITY OF CHEMICALLY INDUCED MURINE TUMORS

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Although there is extensive cytogenetic evidence to suggest that many solid neoplasms may be monoclonal, the uniformity of antigen expression and specificity among the cells of a single neoplasm remains uncertain. The antigenic heterogeneity of carcinogen induced tumors will be investigated by isolating clones from a primary nodule and from a sixth passage transplant generation of methylcholanthrene induced tumors. Clones will be maintained by serial passage in vivo and in vitro.

Resultant pedigrees will be assessed for 1) in vivo growth rate, 2) immunogenicity in syngeneic hosts, 3) suitability as target tissue in specifically immunized hosts, 4) source for extraction of immunoprotective tumor specific transplantation antigens, and 5) contribution to tumor progression in an antigenic competition model. Extracted tumor antigens will be purified by preparative isoelectric focusing. Immunoprecipitated antigenic preparations from non-cross-reactive individual clones will be compared by SDS-polyacrylamide gel electrophoresis. Based upon growth properties and antigenic specificity, selected clones will be admixed and reintroduced into the host in a protocol to study the role of antigenic competition in tumor progression.

Isolation and elucidation of the chemical nature of murine tumor specific transplantation antigens (TSTA) may be impeded by the possibility that these neoplasms are comprised of an array of cells bearing different antigens. Additionally, heterogenic antigen specificity within a single tumor suggests that specific immunotherapy of neoplastic disease may require induction of resistance to all or most of the clones of the primary lesion. It is anticipated that this investigation may provide 1) cell lines to serve as a rich source for large amounts of homogeneous TSTA and 2) a model to study the role of TSTA heterogeneity in the progression of neoplastic disease.

70. IMMUNE RESPONSE TO MEMBRANE ASSOCIATED ANTIGENS OF CHEMICALLY INDUCED RAT HEPATOMAS

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OBJECTIVE: Analysis of the tumor associated immune response in rats bearing chemically induced hepatomas, with the specific aim of establishing the nature and heterogeneity of tumor associated membrane antigens giving rise to formation of both IgM and IgG antibodies during different phases of tumor growth.

APPROACH: Antibodies from tumor bearer sera or from immunized rats are assayed for complement or lymphocyte dependent cytotoxicity to a panel of chemically induced hepatomas differing in antigenicity. Immunochemical and biochemical analyses of plasma membranes from hepatomas or normal hepatocytes and tests of purified antigen fractions for inhibitory activity in cytotoxicity assay are carried out.

PROGRESS: Rat hepatomas D23, D33, D23/Not, D30, D202 (from Dr. R.W. Baldwin, England) were studied for susceptibility to antibody complement mediated lysis by a 51Cr-release assay. The cytotoxic activity in D23 tumor-bearer serum (TBS) was caused by IgM antibodies. Cross-tests of TBS from rats with the different tumors divided the tumors into two groups. Group 1 hepatomas (D23, D33, D23/Not) all shared antigens which could induce serum antibodies in the syngeneic hosts. The group 2 hepatomas (D30, D202) were not lysed in the assay, but induced cytotoxicity against the group 1 tumors indicating the presence of tumor associated antigens shared between the two groups but differing in surface expression. Absorption of a group 1 TBS (D23) with 3M KCl extracts of D23 hepatoma and various tissues inhibited the cytotoxic activity, indicating the autoimmune nature of this immune response. Chromatographic separation of tissue extracts showed that the inhibitory antigen(s) were confined to the high molecular weight fractions (greater than or equal to 200,000).

71. DRUG INDUCED IMMUNOGENIC CHANGES IN TUMOR CELLS

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It has been shown that an antineoplastic drug, 5(3-3'-dimethyl-triazeno)imidazole-carboxamide (DTIC) can mediate the appearance of novel transplantation antigens (drug-mediated tumor antigens, DMTA) on experimental tumor cells. These DMTA have been found to be stable and inheritable. The results of the studies conducted in July-December 1979, supported by a CNR contract, can be summarized as follows: A) Mechanism of DMTA induction. Several hypotheses have been proposed to explain the immunogenic changes induced by DTIC. Mechanisms based on the formation of DTIC-protein complex on tumor cell membrane or on the emergence of naturally occurring, highly immunogenic clones were ruled out. The most attractive hypothesis seems to be related to a DTIC mediated somatic mutation leading to the appearance of new surface protein(s) coded for by mutant gene(s). This hypothesis has been reinforced by the present studies, showing that Quinacrine, a well known antimutagenic compound, impaired the appearance of DMTA in murine lymphoma cells. It was found that Quinacrine antagonizes selectively the immunogenic changes mediated in vivo by DTIC in L1210Ha leukemia cells, whereas it does not affect the antineoplastic or the immuno-depressive activity of the agent. B) Antigenic properties of DTIC-treated tumor cells. DTIC-treated sublines of murine lymphomas were capable of eliciting strong primary cell-mediated responses in vitro. Sensitized lymphocytes were highly cytotoxic for the same DTIC-subline used as stimulator cells, minimally active or inactive against the parental line or other DTIC-treated lymphomas. The extent of cytolytic activity of effector cells was comparable to that obtainable in an allogeneic system. Evidence has also been obtained that this primary cytotoxic response in vitro is T-dependent since it is abrogated by anti-Tyl serum plus complement and cannot be obtained using responder spleen cells of athymic mice of BALB/c background.

72. DEVELOPMENT OF AN ISOTOPIC ASSAY FOR DETECTION OF TUMOR SPECIFIC ANTIGEN ON RAT TUMORS INDUCED BY CHEMICAL CARCINOGENESIS

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OBJECTIVE: Detection of tumor specific antigen and alloantigen by using an isotopic antiglobulin reagent in chemically induced rat tumor. By using 125I F(ab')2 fragment of sheep anti-rat IgG this kind of analytical assay will be combined with a radioimmuoassay of immunoglobulin to measure on a more quantitative basis the antigenic site of tumor cells.

73. IMMUNE MECHANISMS INVOLVED IN TUMOR METASTASIS

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Our studies are intended to clarify some of the mechanisms that operate in tumor metastasis. We are investigating the role of membrane-associated components of tumors, including tumor associated antigens, tumor specific antigens and tumor specific transplantation antigens. We are also investigating the role of specific anti-tumor antibodies and of tumor specific immune complexes. We are studying the interactions between these membrane-associated and humoral components and the various immunocompetent cells involved in the immune response to tumors. Such studies have the ultimate objective of providing new information that can be applied to the prevention or cure of metastatic disease.

For these purposes we are employing a relevant animal model -chemically induced, transplantable rat mammary carcinomas, both metastatic and nonmetastatic. In initial studies we have identified and quantitated a common organ specific tumor associated antigen, apparently characteristic of our metastatic tumors. This antigen is shed by the tumors and may be functionally involved in the metastatic process.

The components of interest are being identified and isolated by specific methods which include the use of immunoadsorbents, lectin affinity adsorbents and xenogeneic antibodies specific for the various membrane-associated components. Sensitive and specific radioimmunoassays are being developed to quantitate these components. Similar sensitive assays are being developed to both detect and quantitate specific anti-tumor antibodies and the specific immune complexes.

A prime objective of these studies is to determine how, and to what extent, the various immunologically defined components are involved in the metastatic process. A second objective is to use the specific analytical procedures we are developing in the animal system as guides in developing procedures for early detection of human tumors.

74. CELLULAR MECHANISMS IN TUMOR-SPECIFIC IMMUNITY

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This project continues studies aimed at understanding host immune mechanisms involved in the tumor-host relationship. The models to be examined involve cloned MCA induced murine fibrosarcomas in multiple congeneic and/or recombinant lines. These tumors are characterized for TSTA, H2 antigens, and MuLV gp70 representation. More information will be sought on the relation between TSTA and H2 or MuLV antigens on the tumor surface. Through immunogenetic and serologic approaches, analysis of H2 deficient varients, and biochemical dissection of the tumor cell membrane, any molecular identities of these elements will be determined. The host responses to tumor bearing will continue to receive scrutiny, in terms of T-cells and T-cell subclasses of known phenotypes, and natural killer cells. Tumor-directed specificity and in vivo as well as in vitro functions as suppressors and killer cells will be delineated. Several techniques developed in this laboratory will be employed in these studies, including TAA-specific proliferation assay, adoptive protection assay, an apparently TSTA-specific cytotoxicity assay, and a tumor cell membrane tagging method.

75. INDUCTION OF TUMOR RESISTANCE WITH BACILLUS CALMETTE-GUERIN (BCG)-ASSOCIATED TUMOR ANTIGENS

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OBJECTIVES: (1) To examine the immunogenicity of soluble tumor antigens coupled to living BCG; and (2) to examine mechanisms of in vivo resistance to tumor growth to assess the capacity of tumor antigen-BCG complexes to provoke protective immunity.

PLAN: Tumor antigen isolated from methylcholanthrene (Mc)-induced sarcomas and coupled to BCG will be tested for capacity to provoke resistance to tumor growth and examined for the kind of anti-tumor immune response induced in syngeneic Wistar rats.

METHODOLOGY: Tumor antigens will be isolated from tumor extracts by electrofocusing and affinity chromatography, and will be coupled to living BCG by incubation with organisms in aqueous medium. Rats receiving tumor antigen-BCG complexes will be evaluated for tumor resistance by direct tumor challenge. Adoptive transfer of lymphocytes will be used to compare the immune response induced by tumor antigen-BCG complexes with that induced by immunization with irradiated tumor tissue. Lymphocytes able to mediate tumor resistance will be characterized by testing subpopulations of cells isolated by affinity chromatography.

FINDINGS: Thoracic duct lymphocytes from rats immunized with irradiated Mc-induced sarcomas mediate specific tumor resistance in vivo. Mediators appear to be T cells that are in a non-proliferating state when recovered from central lymph, and appear to have a minimum effective life span of two weeks. Their mode of activity is unclear but appears to require mitosis before mediating anti-tumor activity.

CLINICAL RELATIONSHIP: This project examines a possible clinically acceptable mode of inducing host resistance to tumor, and the mechanisms of the immune resistance operative in vivo; both aspects are important in clinical immunotherapy of cancer.

76. THE INFLUENCE OF CHEMICAL CARCINOGENS ON VIRAL GENE EXPRESSION IN MAMMARY TUMOR DEVELOPMENT (ABBREV)

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SCOPE: The main objective of this research is to determine if chemical carcinogens (non-viral) that are known to induce mammary neoplasms in mice do so by modification of murine mammary tumor virus (MuMTV)-gene expression, or by acting by an MuMTV-independent alternate pathway.

In the proposed project, the Contractor will study longitudinally, in BALB/C mice, three parameters of the influence of chemical carcinogens, and of chemical carcinogens combined with prolonged hormonal stimulation (pituitary isografts), on MuMTV-gene expression. The three parameters are: (1) influence level (determine the amounts of MuMTV-RNA detectable in mammary gland cells at varying intervals after onset of the oncogenic stimuli); (2) influence of oncogenic stimuli on MuMTV-gene expression at the post-translational level (study alterations of amounts of MuMTV-antigens detectable on cells of mammary gland tissue in host serum, milk, or as antigen-antibody complexes deposited in the kidney); (3) influence of oncogenic stimuli on MuMTV-gene expression as detectable by alterations in the autogenous humoral immune response of hosts to MuMTV-polypeptides, to antigens of murine leukemia viruses, and to non-MuMTV-associated mammary tumor antigens. The oncogenic stimuli used will be prolonged hormonal stimulation, 7, 12-dimethylbenz(a)-anthracene, ionizing irradiation and/or N-nitrosomethylurea. The BALB/C mice will originate from the investigator's own breeding colony.

77. RADIATION AND CHEMICAL EFFECTS ON VIRAL TRANSFORMATION AND TUMOR ANTIGEN EXPRESSION

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The objective of this research is to define the role of low level irradiation combined with DNA containing viruses known to exist in the human population to determine the potentiating effects of irradiation on virally induced neoplasms. The second aspect of the work is to define the role of low levels of chemical carcinogens, particularly hydrocarbons, in potentiating the oncogenic properties of viruses. The primary focus of a large portion of the work will center around determining whether or not it is possible to detect the earliest signs of virally or chemically induced tumors, potentiated by hydrocarbons or irradiation through the monitoring of cells or the immune response of the host to tumor associated antigens which appear to be of embryonal or fetal origin. The approach being used is to detect the earliest transformed cells that appear in cultures of cells exposed to cocarcinogens as reflected by their ability to grow on an unusual test culture surface, polyacrylamide, which seems to have the unique property of excluding the growth of normal cells while potentiating the growth of cancer cells. Cells which become adherent to the acrylamide will be monitored for the expression of embryonic antigens which are common to tumors of the same histologic type. The results expected should allow us to determine whether embryonal or fetal immunologic markers can be used to detect the earliest evidence of chemically or virally induced carcinogenesis which can be potentiated by low level irradiation. We anticipate that our results will be in hand in the coming three years.

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III. TUMOR VIRUS-ASSOCIATED ANTIGENS

A. MTV ANTIGENS

78. MURINE VIRUS CROSS-REACTING ANTIGEN IN HUMAN TISSUE

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We propose to study the specificity, nature, distribution and significance of the antigen(s) in human mammary cells which cross-react with antibody to mouse mammary tumor virus. We will determine the stability of the antigen(s) in mammary epithelial cells cultured from breast tissues and breast fluid

aspirates. Expression of the antigen(s) will be correlated with staging of the breast disease and prognosis and history of the tissue donor. We will attempt to amplify the antigen by manipulation of nutritional milieu, cell interactions and substrate composition with the ultimate objective of biochemical analysis of the antigen.

79. STUDIES ON SOLUBLE ANTIGENS FROM MTV INDUCED MAMMARY TUMOR

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The purpose of this study is (a) to isolate tumor-specific and virus-related antigens from a crude perchloric acid extract of MTV-induced mammary tumors of BALB/cfC3H mice, (b) to assess the biological activities of the isolated components, (c) to determine the cell types involved in the DNA synthetic response of tumor bearing mouse spleen cells to components of the extract, (d) to determine the changes of T cell, B cell and macrophage population with growth of syngeneic transplants of the tumor, (e) to determine the effect of tumor growth on DNA synthetic activity, (f) to determine the nature of serum factors inhibiting the DNA synthetic response and to isolate the serum inhibitory factors, (g) to determine the mechanism of action of the serum inhibitory factors, and (h) to determine the effect of surgical removal of the tumor on lymphocyte response to the tumor extracts, on the changes in T cell, B cell and macrophage populations, and on serum inhibition of DNA synthesis.

80. ANTIGEN SPECIFIC NK ACTIVITY AND SUPPRESSION IN MMTV

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Our overall goal in these studies is to investigate the mechanisms involved in the development of MMTV-specific natural killer cell reactivity and to determine what factors control the decline of this reactivity following spontaneous tumor development in the host neonatally infected with MMTV. We have investigated both nude (athymic) and conventional mice and have found both to possess substantial levels of antigen-specific NK activity to MMTV gp52 and p28. This reactivity declines sharply following spontaneous tumor production. We wish to determine the lineage of the NK cells both in terms of antigen commitment and end point differentiation, and to determine whether there are previously undetected subpopulations of NK cells which recognize 'transformation' antigens in addition to viral antigens.

81. CELL MEDIATED IMMUNITY IN MOUSE MAMMARY TUMOR MODELS

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Three different lines of BALB/c mice are being studied in connection with their incidence of spontaneous mammary tumors (SMT), their expression of mouse mammary tumor virus (MMTV) particles and/or antigens, and their immune response. These lines are: 1) BALB/cCrgl which has a low incidence of SMT, is devoid of intact MMTV but expresses some viral antigens and an in vitro immune response to these antigens as well as to tumor antigens of their syngeneic chemically induced mammary tumors; 2) BALB/cfC3H which has a high incidence of SMT and in which MMTV particles can be detected and 3) hereditarily asplenic BALB/c mice heterozygous for the nude gene, which have a high incidence of SMT (45-50%) with a mean age of appearance of 10 months. The proposed research intends to identify, characterize and isolate the immunologically relevent cells known to respond to either viral or tumor antigens and to use them in adoptive transfer experiments. Studies will include the nature of cellular and humoral in vitro reactions and the possible role of suppressor cells. This immunotherapeutic approach aims at achieving protection of mice with a high risk of developing SMT, thus providing insight into the mechanism(s) operative in tumorigenesis.

82. MMTV IN SPLEEN LYMPHOID CELLS - IMMUNOLOGIC EFFECT

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Mouse mammary tumor virus (MMTV) is expressed in splenic B cells of several mouse strains, as evidenced by immunological means, as a cell surface membrane antigen. We plan to study this antigen(s) in terms of its relation to virion polypeptides, the RNA encoding for its translation and, importantly, whether endogenous or exogenous MMTV genomic sequences encode for its expression. These parameters will be evaluated in low and high incidence breast cancer strains throughout the lifespan of the animals. We hypothesize that MMTV infection of splenic B-cells may be an important factor in the immunologic responsiveness of the host to viral-associated tumor antigens and to the natural immunity of hosts. Using various preparations of the cell membrane antigen we will evaluate immunologic responsiveness using both humoral and cell-mediated immunoassays. If evidence of a significant role in tumor immunity is found, the cell membrane antigen or cells will be used to manipulate host responses to relevant viral-associated breast tumor antigens.

83. ANTIGEN SPECIFIC NATURAL KILLER CELL REACTIVITY AND SUPPRESSION IN MICE NEONATALLY INFECTED WITH MMTV

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Mice neonatally infected with mouse mammary tumor virus (MMTV) are capable of antigen specific natural killer (NK) responses. As we have previously demonstrated, prior to 16 weeks, the C3H/HeJ female possesses natural killer cells capable of recognizing MMTV gp52 ad MuLv gp71. After 16 weeks of age, a response to MMTV p28 is also present. These responses may be detected in vitro with the use of tumor target cells which express these antigens, and may be blocked in vitro by the addition of specific purified viral proteins and glycoproteins. Females of this strain bearing spontaneous mammary tumors of 15x15 mm or greater, demonstrate reactivity only against MuLv gp71. The spleens of these tumor-bearing females possess a cell capable of specifically suppressing NK responsiveness to antigens of MMTV, but no MuLv. We propose to investigate the significance, in this strain, of the arising of an MMTV p28 response and its relationship to antigen expression of preneoplastic nodules: to define the precise timing of decline of immune responsiveness to MMTV gp52 and p28 during tumor development; and to investigate the nature of the antigen-specific suppressor cell described in this system. Based on findings in these studies we will institute modes of therapy with purified antigens of MMTV. These treatments will be designed to alter pre-existing host levels of immune responsiveness to benefit the host in the prevention of tumors.

84. DETERMINATION OF THE TISSUE TROPISM FOR ACQUISITION OF NEW PROVIRUSES FOLLOWING CONGENITAL INFECTION OF MICE WITH MUMTV

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A limited exposure to virus-containing milk at 1-2 days of age results in the overt infection of the mouse mammary epithelium in adult life. Infection of other tissues with MuMTV has not been established, although the presence of viral antigens and the occasional budding virus particle have been reported. However, investigations into the presence of MuMTV antigens following congenital infection have been subject to some ambiguity resulting from the fact that all inbred mouse strains examined contain endogenous viral sequences which could be expressed. The provirus of the endogenous and exogenous MuMTVs can be distinguished on the basis of the specific DNA fragments arising from each provirus following endonuclease digestion. As little as 10 percent infection of a particular tissue can be detected on the basis of the pattern of restriction endonuclease-cut DNA fragments, visualized following agarose gel electrophoresis, blotting and hybridizing with specific viral DNA probes.

This method has the advantage that no expression of the virus is required for detection of the provirus. An examination of the DNA from tissues of infected mice for the presence of new proviruses should help to elucidate the route of viral infection and the extent of the tissue tropism. Preliminary results indicate that the congenitally acquired MuMTV was not present at a detectable level in the liver, kidney, spleen or brain of infected animals.

85. MAMMARY TUMOR VIRUS GLYCOPROTEINS AND ANTIGENS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

86. MTV-ASSOCIATED ANTIGENS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

B. AVIAN LEUKEMIA AND SARCOMA VIRUS-ASSOCIATED ANTIGENS

87. AVIAN LEUKOSIS VIRUSES AND CANCER

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Avian leukosis viruses (ALVs) which have defined genetic compositions will be used to examine the role of viral envelope antigens and the C region of viral RNA in ALV-associated disease. Viruses which are present in ALV-associated lymphomas will be characterized to determine the relationship of tumor associated viruses to infecting viruses. Novel tumor associated viruses will be identified by examining tumors for novel viral proteins and RNAs. A major effort will be made to culture tumor associated viruses which code for novel proteins and RNAs. Novel viruses will be tested for their oncogenic potential. Our working hypotheses are: (1) Any ALV which actively replicates in a target tissue has the potential of becoming a transforming agent for that tissue. (2) ALVs become transforming agents when they recombine with cellular genes to give viral genes which affect the growth and/or differentiation of tissues. (3) Transforming agents which are formed during ALV infections cause disease in specific target cells after short latent periods.

88. TOLERANCE AND IMMUNITY TO AVIAN RNA TUMOR VIRUSES

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The proposed objectives are: 1. To define the relevant antigens (VEA, TSSA or others) involved in tumor recognition in chickens and to determine the relative contributions of the cellular and humoral limbs of the chicken immune system in tumor growth and rejection in vivo and as assessed by in vitro correlates of immunity. 2. To determine if T cells from RSV and ALV immunized chickens recognize TSSA and VEA of other heterologous ATV subgroups via group-specific determinants. 3. To further investigate the immune competence of birds congenitally infected with ALV. To determine if leukocytes (T and B cells) from birds congenitally infected with ALV fail to recognize VEA or TSSA of homologous and heterologous subgroups and if leukocytes deliberately infected in vitro, as well as in vivo, with ALV can recognize TSSA or VEA. 4. To define the importance of antibody dependent cellular cytotoxicity (ADCC) in birds bearing RSV-induced tumors and in birds infected with ALV and to correlate ADCC with other parameters of the immune response and the outcome of ATV-induced neoplasia. 5. Characterization of the specificity and avidity of normal and ALV congenitally infected chickens' humoral response to whole virus and to purified virion structural antigens.

89. THE IMMUNE RESPONSE TO CELLS INFECTED WITH AVIAN SARCOMA VIRUS

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Experiments are proposed to characterize the immune response to the group-specific and type-specific determinants of the viral envelope glycoprotein in chickens infected with avian sarcoma virus. The induced type-specific or group-specific reactivity will be measured in regressor chf(negative) and chf(positive) chickens as well as in sensitized 72 x 15B chickens. The possibility that tumor-specific antigenic determinants associated with the viral envelope glycoprotein on transformed cells may be immunogenic for helper T cells will be assessed by measuring induced reactivity in chickens sensitized with BH-RSV(negative). The proposed experiments should serve to further test a model developed to rationalize the immunogenicity of the viral envelope glycoprotein.

90. IMMUNOLOGIC PROBING OF SARCOMA DIVERSITY

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OBJECTIVE: In virally induced sarcomas there are four general classes of antigens potentially recognized by the host; these are 1) virion structural proteins, 2) modified precursors to virion proteins, 3) viral gene products not related to virion structural components (i.e., 'sarc' gene products), and 4) host proteins modified by viral gene expression. The basic goal of this proposal is to define in molecular terms the antigens recognized by a sarcoma-bearing host. As a part of this work, we will take advantage of the genetically well-characterized avian system to identify the 'sarc' gene product from a series of sarcoma viruses, and to establish the extent of diversity within this family of molecules.

91. ISOLATION AND CHARACTERIZATION OF AVIAN SARCOMA VIRUS SPECIFIC TUMOR ANTIGENS

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This collaborative project chemically identifies and isolates the avian sarcoma virus (ASV) tumor specific transplantation antigens (TSTA), and other ASV tumor antigens (proteins) uniquely expressed in avian or mammalian cells transformed by ASV. Three basic approaches are employed. First is an immunological approach in which antisera specific for ASV tumor antigens are prepared. Second is a chemical approach in which specific protein components which are expressed in ASV transformed - but not in normal - cells are defined. There is a biological approach based on hypothesized functions for ASV specific tumor antigens, e.g., binding to single- or double-stranded cellular DNA, or isolation of specific protein components unique to ASV transformed cells which function as TSTA in vivo transplantation tests.

The basic chemical structure, subcellular localization, metabolism, and biological function of ASV specific tumor antigens are being determined. These studies utilize mammalian and avian cells infected (or transformed) with wild-type and also mutant ASV to determine whether ASV tumor antigens are virus encoded, and also to attempt to clarify the primary role of these tumor antigens in the transformation process.

92. AVIAN SARCOMA VIRUS TRANSFORMATION-SPECIFIC ANTIGENS

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The goals of this supplemental application remain the same as those outlined in our initial application. Those objectives as originally stated in June of 1976 are reproduced here.

This study is directed toward the identification of avian sarcoma virus (ASV)-coded non-virion proteins. Antiserum from mammalian hosts which carry ASV-induced tumors will be used to identify virus-specific and transformation-specific antigens in ASV-infected chick cells. To date we have obtained hamster antiserum which will specifically immune precipitate 35S-methionine-labeled antigens form ASV-transformed chick

cells which correspond to virion internal group-specific antigens and their precursors, plus antigens not previously reported in infected cells. Antiserum will be produced in several other species in order to ensure the greatest variety of immunological responses.

These studies will be extended using ASV-transformation defective and temperature sensitive mutant-infected chick cells in order to identify those antigens that are transformation specific. If such a protein(s) is identified, it will be purified and its intracellular location determined. In this way, we hope to deduce how such a protein(s) functions.

Now that the product of the ASV src gene has been identified, the major emphasis in our laboratory has shifted to its purification and studies on its mechanism of action. However, many of the original experiments proposed are still incomplete.

We will utilize: 1) immunoprecipitation techniques, 2) enzymatic and nonenzymatic degradation in order to characterize proteins, 3) ion-exchange and immunoaffinity chromatography, and 4) protein kinase assays.

93. IMMUNOLOGICAL ASPECTS OF THE HOST-TUMOR RELATIONSHIP IN CHICKENS BEARING TUMORS INDUCED BY ROUS SARCOMA VIRUS

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OBJECTIVE: We wish to further characterize the antigens responsible for the elucidation of humoral and cellular immunity against avian sarcomas, and to determine whether the immune system plays a major role in causing tumor regression in this model.

APPROACH: A variety of immunological methods, including cell-mediated cytotoxicity, antigen-driven lymphocyte blastogenesis, indirect immunofluorescence and immunoprecipitation, are used to monitor for concomitant antitumor immunity. At the same time, cultured avian sarcoma cells are tested for ability to produce complete or defective progeny virus particles.

PROGRESS: We have determined that the humoral response of tumor-bearing chickens is directed almost exclusively against the major viral glycoprotein, gp 85, while cellular responsiveness can also be detected against a group specific, transformation-independent, non-virion moiety. In addition, recent experiments reveal that tumor cells which are cultured from regressing tumors can produce glycoprotein-deficient virus. We now wish to determine the mechanism of shut-down of transforming virus production in these cells and feel that this phenomenon may be triggered following immune system involvement.

94. IMMUNOLOGICAL AND STRUCTURAL PROPERTIES OF RSV TRANSFORMED FIBROBLAST PLASMA MEMBRANES

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OBJECTIVES: (a) Isolation and biochemical characterization of the membrane molecule(s) responsible for the VCSA antigenic specificity. (b) Studies on the immune response to the VCSA, EA and VEA in mice bearing RSV induced sarcomas.

APPROACH: (a) Production of monoclonal antibodies against surface antigens expressed by RSV transformed fibroblasts and (b) immunization of mice with cells transformed by RSV mutants either VEA (negative) or VCSA (negative).

PROGRESS: Previous work has shown that Rous sarcoma virus (RSV) transformed fibroblasts of different animal species express, at the cell surface tumor membrane, antigens of three different genetic origins and specificities: (a) VEA (virus envelope antigens) coded by viral structural genes, (b) EA (embryonal antigens) due to the depression of silent cell genes, and (c) VCSA (virus cell surface antigens), tumor specific antigens controlled by the viral src gene whose sole function is induction and maintenance of the transformed phenotype.

95. THE IMMUNE RESPONSE TO TUMOR ANTIGEN INDUCED BY ROUS SARCOMA VIRUS

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This is part of a broader project. A summary of this subproject is not available.

96. IMMUNOGENETICS OF TUMOR RELATED ALLOANTIGENS

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Host-cell susceptibility to the leukosis-sarcoma viruses is dependent on specific interactions between genetically controlled properties of the viral envelope and the host-cell plasma membrane. The cell membrane 'receptor' may act as an inherited alloantigen and be detectable by existing serological methods. One objective of this proposal is aimed at the serologic identification of erythrocyte alloantigens which may be associated with any of three different autosomal loci, each controlling susceptibility to a different viral subgroup - A, B, or C. A large colony of genetically variable chickens segregating for twelve systems of erythrocyte alloantigens and the three viral receptor loci will serve as appropriate recipients and donors to elicit anti-receptor hemagglutinins and/or lymphoagglutinins for one receptor and for as few regular erythrocyte alloantigens as possible. The already demonstrated association of R1 erythrocyte alloantigen with susceptibility to the B subgroup of viruses indicated that it should be possible to elicit allohemagglutinins for the A and C subgroups as well.

Another primary objective is the investigation of the substructure of the alloantigen-determining portion of the B histocompatibility complex. The significance of this investigation rests on recent findings that certain B haplotypes either inhibit specific tumor formation or are very effective in initiating tumor regression. Recent evidence suggests that recombination within the B complex may be in the one to two per cent range which should allow for adequate recovery of recombinants under an efficient immunogenetic experimental design.

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C. OTHER LEUKEMIA AND SARCOMA VIRUS-ASSOCIATED ANTIGENS

97. AFFINITY STUDIES ON NATURALLY OCCURRING ANTIBODIES TO TYPE C VIRAL ANTIGENS IN MAN

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Studies on the affinity of naturally occurring human antibodies to the simian sarcoma SSV(SSAV) and Friend leukemia virus (FLV) envelope antigens have been continued and extended (see ICRF Scientific Report, 1978 report, 307).

The relative affinities (Kr, liters/mole) of the antiviral SSV(SSAV) and FLV antibodies were determined in about 60 sera, both normal and pathological, using a double antibody precipitation method which was a modification of that described by Gaze et al, Journal of Immunological Methods, 1973, 3, 357. The samples chosen were those giving high levels of binding to both antigens in direct radioimmunoassay. About 40 percent reacted as high affinity sera with binding constants between 2.4 times 10 to the 9th power and 2.0 times 10 to the 8th power liters/mole for the SSV(SSAV) gp 70 and 1.4 times 10 to the 9th power and 1.3 times 10 to the 8th power for the FLV gp 71. Kr values for the SSV(SSAV)-related antibodies were consistently higher than those of their FLV counterparts, indicating a closer homology with the SSV(SSAV) glycoprotein. Sucrose density gradient centrifugation of complexes formed between a high-binding serum and 125I SSV(SSAV) or FLV antigen showed small, but antigen-specific, complexes present in both cases.

No apparent correlation of the highest affinity sera with any specific disease group was observed for either antigen, some of the highest Kr values were found in serial serum samples from a laboratory worker and probably resulted from a long term exposure to the SSV(SSAV) virus. The preferential reactivity of the human antibodies to the viral envelope antigen rather than to the internal proteins parallels that seen in cats and mice and indicates that the human antibodies probably bind to a subset of antigenic sites on the viral envelope shared by viruses

from different host species, the so-called 'interspecies determinants'.

98. IMMUNOLOGICAL RELATIONSHIPS BETWEEN HUMAN LEUKEMIA ASSOCIATED ANTIGENS AND RNA TUMOR VIRUSES

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The goal of this research is to determine the nature of the virion and cell associated antigens of HL23 virus detected by chimpanzee and goat antisera. Using cytotoxicity, RIP, PAGE, and absorption techniques, these antigens will be compared with analogous antigens of other primate C-type viruses. Other studies are designed to determine serological reactivity of chimpanzee and goat antisera to HL23 virus with antigens of human leukemia and lymphoma cells.

99. FELINE LEUKEMIA VIRUS (FELV) ANTIGENS AND ANTIBODIES AGAINST FELINE ONCORNAVIRUS ASSOCIATED CELL MEMBRANE ANTIGEN (FOCMA)

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OBJECTIVES: 1. Examination of horizontal transmission of FeLV in cats, as a basis for epidemiology and for vaccination programs. 2. Determining whether FeLv plays any role in the etiology of lymphosarcoma/leukemia in man.

APPROACH: 1. Feline and human blood smears are examined by IFA-test on the presence of FeLV-antigens. 2. Feline and human sera are examined by Proteline-A (solid face) test, radioimmunoprecipitation test, fluorescence, neutralization, and FOCMA-test on the presence of FeLV or FOCMA antibodies. 3. Diverse types of killed FeLV-vaccines are used in 14 week old kittens.

PROGRESS: A removal program has been designed especially for catteries and so called multiple-cat households in an effort to control lymphosarcoma/leukemia in cats and transmission of FeLV was set up. The removal program calls for: annual testing of all stud cats; testing of all contacts of a FeLV-positive cat during the previous 2 years; testing of imported cats; and isolation of FeLV-positive cats or, if this is impossible, euthanasia. The FeLV-antigen can be demonstrated in cats by the indirect immunofluorescence antibody (IFA) test. A removal program has been carried out now over a 4-year period in a large Dutch cat club. The percentage of FeLV-positive catteries decreased from 11.5% during the first half of 1974 to 2.1% during the latter half of 1977; during the same period the percentage of FeLV-positive cats and stud cats decreased respectively from 4.9% and 5.9% to 1.2% and 1.0%. In our vaccination program 16 cats are vaccinated i.m. during week 11 and 13 with 2 different doses (1 or 2 mg virus protein per injection) of 2 different vaccines. Vaccine I is an antigen-antibody complex (FeLV inactivated by formaldehyde, bound with rabbit anti-FeLV-serum). Vaccine II is an FeLV (killed)-Sepharose complex. Both are injected together with Freund's incomplete adjuvants. The antibody titers against FOCMA after vaccination vary from titer 2:512 using FI-74 cells as targets and from titer 2:16 using non-producer mink cells transformed by FeSV as targets. No neutralizing antibodies could be detected during week 15. Six weeks after vaccination all cats were challenged i.p. with 5 x 10 to the 5th power f.f.u. of FeLV-1-strain. (Text Abridged.)

100. CHEMICAL NATURE OF LEUKEMIA ANTIGENS

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We propose to characterize feline leukemia cell surface proteins associated with feline leukemia virus. The nature of cell surface molecules reactive with antisera to individual viral structural proteins will be determined. The reactivity of feline sera with such cell surface proteins will also be analyzed. Sera from healthy cats exposed to the virus in the natural environment, exposed cats which develop lymphosarcoma, and unexposed cats will be surveyed. Of particular interest will be characterization, on naturally occurring lymphosarcomas, of the feline oncornavirus-associated cell membrane antigen (FOCMA) which appears to be a tumor-specific antigen. Purification of FOCMA-carrying molecules will be attempted, as a step toward development of a vaccine against feline leukemia. In parallel studies we will compare the nature of FOCMA with that of human acute lymphocyctic leukemia cell antigens reactive with autologous antibodies from patients' sera.

BIBLIOGRAPHIC REFERENCES: Snyder, H.W., Jr., K.J. Phillips, W.D. Hardy, Jr., E.E. Zuckerman, M. Essex, A.H. Sliski and J. Rhim. 1979. Isolation and characterization of proteins carrying the feline oncornavirus-associated cell membrane antigen (FOCMA). Cold Spring Harbor Symp. Quant. Biol. XLIV., in press.

101. FOCMA AND ITS THERAPEUTIC APPLICATION

Theilen, G. H., Troy, F. A., Pedersen, N. C., Madewell, B. R., University of California, Davis Campus, School of Veterinary Medicine, Dept. of Surgery, *Davis, California*, 95616, United States of America

OBJECTIVES: 1) Isolate and characterize feline oncornavirus-associated cell membrane antigen (FOCMA). FOCMA will be isolated by using polyacrylamide gels and triton X-100 extraction. Competitive cytotoxicity will be used for in vitro detection of FOCMA. 2) Study the role of FOCMA as an immunogen in the prevention of neoplasms in cats. Antibodies to FOCMA are known to be tumor protective in cats that have recovered from feline oncornavirus infections. There is evidence that FOCMA antigens are present in FeLV and non-FeLV induced neoplasms of cats. Similar molecular weight proteins have been isolated from the cell membranes of avian and rodent tumors and these antigens are protective against oncornavirus and chemically induced neoplasms. Cats will be vaccinated with FOCMA antigen(s) to determine its efficiency as an immunoprevention of cancer. If FOCMA evokes anti-tumor immunity, it also has obvious value as a model in immunotherapy.

102. IMMUNOPREVENTION OF CANCER IN CATS

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OBJECTIVES: To determine the interrelationship of RD-114, FeLV and src gene expression to FOCMA antigen in naturally occurring and chemically induced feline cancer. To determine if transformation-specific scr genes can be rescued as sarcoma viruses from cat tumor cells. To determine the relationship between FOCMA and the hypothetical feline src gene transforming protein. To determine the correlation between autogenous immunity to FeLV or FOCMA and occurrence of disease. To determine whether cats can be protected against natural or induced cancer by immunization with FOCMA and/or feline transforming protein. To determine whether humans show any evidence of infection after exposure to FeLV.

103. ANTIGENICITY OF PRECANCEROUS LESIONS IN ANIMAL MODELS

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OBJECTIVE: Study the sequential development of feline oncornavirus-associated cell membrane antigen (FOCMA) in preleukimic bone marrow cells in cats infected with feline leukemia virus (FeLV).

APPROACH: The principal model used is the Rickard strain of FeLV in SPF cats. This study will attempt to ascertain when FOCMA appears in preneoplastic cells in relation to other markers associated with the preleukemic phase of the disease (i.e., suppressed T-cell function, induction gsa in FeLV infected cells, lymphophenia). The shedding of FOCMA from preneoplastic and/or neoplastic cells will be determined by either radioautography, or radioimmunoprecipitation or by cytotoxicity-inhibition assays.

104. IMMUNE RESPONSE IN LEUKEMIA

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In previous studies we demonstrated that an efficient immunosurveillance mechanism ordinarily protects cats against development of leukemia-lymphoma following natural exposure to the feline leukemia virus (FeLV). This immune response is not

directed to the FeLV itself, but rather to the feline oncornavirus-associated cell membrane antigen (FOCMA) which is probably the clearest example of a RNA tumor virus encoded tumor specific antigen. The humoral antibody response to FOCMA is clearly associated with protection against tumor development, and the antibodies to FOCMA are highly lytic in the presence of cat complement.

We plan to test our 'immunoselection hypothesis' which proposes that 'virus-negative' tumors of cats (and possibly other species) may result from immune responses that are discordant between anti-viral and anti-tumor components. According to this hypothesis, an efficient anti-FLV gp 70 response in the absence of an adequate anti-FOCMA response would select for tumor clones that do not make virus. Other possible immune effector mechanisms, particularly NK activity, ADCC, and T cell immunity would be evaluated with the humoral response in a comparative sense using FOCMA on tumors of lymphoid, fibroblastoid (sarcoma), and epitheliod (melanoma) tumors as targets.

Studies on the significance of histocompatibility antigens in virus neutralization will be continued in the human HLA (cell culture) system and initiated in the cat model. The NK system in the cat will be studied in relation to both FeLV and FOCMA targets, including the relationship of this system to genetic background and potentiation by interferon. Studies of mechanisms by which RNA tumor viruses interfere with the immune response will be continued. Of high priority will also be studies on the nature of the lymphoid cell target in the cat and the effect of the virion protein p15e on various parameters of lymphoid cell function. The antigenic nature of immune complexes observed in cases of persisting natural infections with FeLV will be evaluated.

105. SEROEPIDEMIOLOGY OF SPONTANEOUS FELINE LEUKEMIA

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For the past years we have been studying cats with naturally occurring leukemia, lymphoma, and fibrosarcoma. This system is uniquely valuable for seroepidemiologic, immunologic, and virologic studies. In the past few years, for example, these studies led to observations (a) that the feline leukemia virus (FeLV) is transmitted horizontally, (b) that a natural immunosurveillance mechanism works to control against tumor development by these viruses, and (c) that the target of this immunosurveillance is a tumor- or transformation-specific non-virion antigen that we call the feline oncornavirus-associated cell membrane antigen, or FOCMA.

In the next two years, we plan to continue these studies, concentrating on the following issues related to the virus itself: (1) What host level and cell level factors determine whether FeLV will actively replicate in the tumor cells? (2) Is the FeLV found in tumor cells different from the FeLV found in normal cells of the same animal or that found in normal healthy animals? (3) Can we rescue FeLV or FeSV from the so-called 'virus-negative' leukemias, lymphomas, melanomas, and fibrosarcomas of spontaneous origin? Our studies will also be directed to immune parameters to determine what role component levels and antibody-dependent cell cytotoxicity may play in the immunosurveillance (along with the FOCMA antibody). A genetic analysis of cats of three breeds and several families (within each breed) will be undertaken to see how these factors might affect tumor and viral immunity and pathologic response.

106. TUMOR IMMUNITY AND FELINE LEUKEMIA

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FOCMA is a transformation or tumor-specific antigen induced by the RNA containing leukemia and sarcoma viruses of cats. In experimental and field conditions FOCMA is the target for an efficient anti-tumor immune surveillance mechanism which prevents the appearance of spontaneous lymphoid malignancies. Immune surveillance operates to prevent appearance of tumors which support virus replication, and also tumors of similar histologic type which lack any evidence of virus infection per se but which nevertheless express FOCMA, i.e., virus positive and virus negative leukemias.

We plan to characterize the immune mechanisms which prevent appearance of cat leukemias. The major assay system used will be the 51Cr release cytotoxicity assay and emphasis will be placed on using assay mixtures which contain only cat components. We will study the activity of all known immunological effector mechanisms and attempt to piece together the interaction between them. As such we will assay for naturally occurring mechanisms, i.e., NK cells and the virolytic effect of complement, as well as immune mechanisms, i.e., cellular cytotoxicity mediated by T cells and/or macrophages, antibody dependent cellular cytotoxicity and ADCC effector cell levels, virus neutralizing antibody, complement dependent antibody and complement levels. The nature and magnitude of these responses in cats will be defined before and after natural exposure to (or inoculation with) leukemia or sarcoma virus, and changes following the onset of persistent viremia or appearance of tumors will be investigated.

In parallel studies we will investigate the quantitative expression and crossreactivity of FOCMA on individual tumor isolates; associations between FOCMA expression and cell cycle; and aspects of the preservation of antigen expression on tumor cells rendered non-viable by various methods. These studies will provide some data necessary for development of an effective FOCMA vaccine against progressive tumor growth in cats.

107. STUDIES ON TUMOR ASSOCIATED CELL SURFACE ANTIGENS AND VIRUS PRODUCTION IN THE MOLONEY MURINE LEUKEMIA VIRUS SYSTEM

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This project consists of two main parts. Part I: Studies on the Moloney leukemia virus (M-MuLV)-determined cell surface antigen (MCSA). 1. Biochemical characterization of MCSA. a) Isolation of gag-protein-MCSA complexes on anti-p30,p15 and p12 immunoadsorbent columns from a series of Moloney lymphoma cells and their immunoresistant sublines. b) Immunoprecipitation with monospecific antisera to viral structural proteins and mouse anti-Moloney lymphoma sera. Isolation of immunoprecipitates on NaDod SO4 polyacrylamide gels. 2. Is MCSA the target antigen for the in vivo rejection reaction against Moloney lymphomas? a) Immunization with electrophoretic fractions of MCSA. b) Transplantation tests with immunoresistant Moloney lymphoma sublines selected in vivo and in vitro on anti-Ig columns. 3. Studies on the appearence and distribution of virally determined antigen in lymphoid organs of mice during leukemogenesis by the M-MuLV. Search for viral recombinants during M-MuLV leukemogenesis by characterizing the tryptic peptides of viruses isolated from various lymphoid tissues.

Part II: Studies on the restrictive effect of the Fv-1 locus on virus production in hybrid cells.

108. MEMBRANE-BOUND ENZYME INTERACTIONS AND GROWTH CONTROL

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The long-term objective of this investigation is directed towards understanding more fully the potential mechanism(s) whereby virus-determined surface antigens influence lymphoid cell proliferation and malignant growth. This objective encompasses studies concerned with the two following interrelated problems: 1. The chemical nature and mechanism of synthesis and assembly of the Moloney leukemia virus-induced cell surface antigen (MCSA) on mouse lymphoma cells and in immunoresistant sublines; 2. the molecular differences in membranes from Epstein-Barr virus (EBV) negative lymphoma lines and their in vitro EBV-transformed sublines.

Current goals are a logical continuation of studies currently in progress which are examining two specific questions: 1. What are the structural changes in MCSA isolated from immunoresistant sublines? 2. What are the specific surface differences which result from the presence of the Epstein-Barr virus in human lymphoma lines?

Since many alterations in the cell surface have been implicated in malignant growth, knowledge of the molecular mechanisms underlying these processes may contribute significantly to our understanding of recurrence and metastasis in human cancer.

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109. STUDY OF LEUKEMOGENESIS IN THE AKR MOUSE

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Extend present serological typing systems to identify new cell surface antigen systems specified by various classes of endogenous MuLV, using techniques developed in the AKR-Gross MuLV system. Characterize new antigens biochemically and determine relationship to viral structural components. Conduct detailed immunogenetic and virological analyses of preleukemic phase of AKR mice. Use genetic approach to analyze age-dependent amplification of MuLV antigenic expression in thymus of six month old AKR mice. Determine biological and biochemical character and significance of MuLV isolated from AKR mice as listed above. Use cell fractionation techniques to identify the cellular source of various classes of MuLV from thymus of different age AKR mice.

110. SPECIFICITY OF T CELL-MEDIATED CYTOTOXIC REACTIONS WITH CHEMICALLY INDUCED MURINE SARCOMAS

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Chemically induced sarcomas of mice have antigens that engender specific anti-tumor immune response that can bring about rejection in preimmunized syngeneic recipients. In contrast to the individually distinct tumor antigens defined by transplantation techniques with these sarcomas, in vitro studies for cell-mediated immunity have sometimes suggested the presence of shared tumor-associated cell surface antigens. However, the lack of critical specificity controls for these in vitro reactions makes interpretation of these studies extremely difficult. The fact that MuLV and its antigens are often found in chemically induced sarcomas, and that normal mice frequently respond to these antigens, has added another serious complication to the study of these tumors. We propose to use in vitro cell-mediated cytotoxicity assays and in vivo Winn assays, as well as information accumulated through serological analysis of these sarcoma cells to define the cell surface antigens of chemically induced tumors that are recognized by cytotoxic T cells. Specificity of cytotoxicity will be analyzed by 1) cellular immunoadsorption tests with a series of syngeneic and allogeneic MuLV positive and MuLV negative fibroblast and sarcoma cell lines grown as monolayer cultures, 2) cellular inhibition assays which test the effects of MuLV positive and MuLV negative cell lines on cell-mediated cytotoxicity in vitro and in Winn assays in vivo, and 3) antibody blocking tests which measure the effects of high specific antibody to tumor-specific cell surface antigens (e.g., Meth A), alloantigens and MuLV-related antigens, on the interaction of cytotoxic T cells with target cells and on cellular adsorption of cytotoxic T cells.

111. TUMOR CELL ANTIGEN CHANGES

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A system has been developed for in vivo and in vitro study of antigenic changes occurring in rat virus-induced leukemia cells under the effect of the immune response. In vivo, leukemia cells cease to express virus-associated antigens when passaged through total-body x-irradiated rats but revert to antigenic expression even after long intervals of serial antibodies. The cycles of antigenic conversion and reversion of leukemia cells will be monitored in vivo and in vitro with the use of indirect immunofluorescence using anti-Gross murine leukemia virus antisera as well as anti-p12, -p30 and -gp70 monospecific antisera. The antigenic modulation will also be periodically monitored by cytotoxicity tests and assays for the presence of reverse transcriptase. Histologic and electron-microscopic studies will investigate the morphologic alterations that accompany the antigenic changes. The oncogenicity and the virus production of modulated leukemia cells will be assayed by cell-free filtrates in susceptible newborn rats. The aim of this project is to study the mechanisms of antigenic modulation of leukemia cells and the correlations with tumor growth.

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112. ANTIGENS OF ABELSON OR FRIEND MURINE LEUKEMIA VIRUSES

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Using serological techniques we have recently identified tumor-specific cell surface antigens induced by the Abelson or Friend murine leukemia viruses. Examination of tissues from uninfected mice has revealed that these determinants are also differentiation antigens of early hematopoiesis. Genetic studies indicate that expression of these antigens in uninfected hematopoietic tissue is controlled by loci which govern susceptibility to viral oncogenesis; namely the Av-2 locus for Abelson antigen and the Fv-2 locus for Friend erythroleukemia antigen.

Our research program will further investigate the role of these antigens in Abelson and FV diseases, and in hematopoietic differentiation. In particular, we will determine more precisely which hematopoietic precursor cells express these antigens through short-term in vitro colony assays, and which loci govern their expression through construction of antigen congenic mouse strains. We will investigate antigen expression in infected genetically resistant animals, and antibody production by resistant and susceptible strains of mice. We also plan to identify antigen-bearing molecules in both virus-infected and uninfected hematopoietic cells using biochemical techniques. Possible functions for these antigens will be investigated by determining their response to hormones involved in hematopoietic differentiation. To facilitate serological detection of these antigens hybridoma lines producing monoclonal antibodies to these determinants will be isolated.

113. IMMUNOLOGICAL ASPECTS OF MOUSE LEUKEMIAS

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Mice of a variety of inbred strains are actively undergoing an immune response to endogenous leukemia viruses (MuLV). This is reflected by the presence of circulating antibodies, in the sera of normal mice, against the gp70 and p15(E) envelope proteins of MuLV. Immune response to MuLV is controlled by two major genetic factors: (1) Genes that control the expression of endogenous MuLV determine the presentation of immunogen to the host; (2) genes in the IXth linkage group, mapping within H-2, determine the ability of the host to develop a secondary immune response to MuLV. Immunization of mice with leukemic cells induces an immune response to the viral envelope proteins gp70 and p15(E), as well as to two glycosylated forms of the gag gene product. Antibodies to these glycosylated polyproteins crossreact with the p30 protein of the viral core.

The purpose of this research project is to investigate further the parameters of immune response to MuLV and leukemia-associated antigens. In the initial phase of this program we will perform a detailed analysis of the humoral and cellular components of natural immunity against leukemias. This analysis will be directed at determining the efficacy of this immune response in controlling the occurrence of spontaneous leukemias. Genetic crosses that segregate for immune response, production of infectious MuLV, and spontaneous leukemogenesis will be used to determine relationships between these phenomena. Subsequent studies will lead to the isolation of viral and cell-associated antigens for chemical analysis and production of

nonspecific antisera. Purified antigens will then be used for immunization of mice to determine immunogenicity and potential protective effects against disease.

114. MECHANISMS OF IMMUNE RECOGNITION OF VIRAL ANTIGENS IN PERSISTENT VIRAL DISEASES

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The major goal of this project is the definition of mechanisms of immune recognition of viral antigens on the cell surface of virus infected cells. We are continuing to study the murine leukemia line FBL-3 which undergoes a major phenotypic alteration in vivo converting from benign to malignant behavior. Cells grown in vitro or for 7 days in the ascites form produce transient tumors when injected subcutaneously. The cells passed in ascites for 14 days produce lethal subcutaneous tumors which metastasize. We have found by fluctuation analysis that malignant tumor cell variants exist in the parent line and that as yet undefined selective pressure in the host allows the preferential expansion of the pre-existing variants. Detailed studies of the surface antigens expressed by cloned tumor cell variants have revealed important aberrations in the major histocompatibility antigens expressed by these cells which may adversely affect their immune recognition by the syngeneic host.

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115. VIRUSES AND THE IMMUNE RESPONSE

Morse, H. C., Chused, T. M., Hartley, J. W., Sharrow, S. O., Goode, J. H., Taylor, B. A., Davidson, W. F., Wolford, N. K., Longstreth, J. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Experimental Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Genetic analyses of xenotropic murine leukemia viruses in inbred mouse strains have shown that these viruses may be expressed as infectious virus or as virus-coded antigens (gp7-'s termed XenCSA) on the surface of normal lymphocytes. NZB mice have two independently segregating dominant loci for infectious virus expression and apparently one coding for XenCSA in the absence of infectious virus. The levels of virus expression as infectious virus and XenCSA in NZB lymphocytes are governed by genes affecting lymphocyte differentiation. In genetic crosses of mice exhibiting high (DBA/2) and low (C57BL/6) levels of XenCSA, a single semidominant gene on chromosome 4 at or near Fv-1 has the predominant effect on XenCSA levels.

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116. GENETICS OF MURINE LEUKEMIA RELATED ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

117. TRANSMISSION OF BOVINE LEUKEMIA VIRUS AND CHARACTERIZATION OF THE VIRION

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OBJECTIVE: Determine how bovine leukemia virus is transmitted among cattle. Study the antigens of bovine leukemia virus and the immunologic responses of cattle to these antigens. Characterize bovine leukemia virus virions. Ascertain any human health hazards posed by bovine leukemia virus.

APPROACH: Gel-diffusion and complement-fixation tests will be used to follow virus transmission in infected dairy herds and the immunologic responses of experimentally infected calves. Proteins and nucleic acids from disrupted virions and infected cells will be collected, purified and characterized using column chromatography, gel electrophoresis and ultracentrifugation. The antigenic activity of these fractions will be determined by complement-fixation and gel-diffusion tests. Human sera will be tested for bovine leukemia virus antibodies and primates will be inoculated to delineate the human health hazards associated with bovine leukemia virus.

PROGRESS: Satisfactory methods have been devised to prepare inactivated bovine leukemia virus vaccines. If these preparations are used with appropriate adjuvants, detectable serological responses are stimulated in vaccinated calves. The effectiveness of these responses in preventing bovine leukemia virus infections has not yet been clearly established. Pathogenesis studies have provided evidence that the spleen is an important early site of virus replication in cattle recently infected with bovine leukemia virus. The absence of evidence of widespread virus replication or a highly viremic state provides additional evidence that the transfer of infected peripheral blood leukocytes from animal to animal is the primary means by which bovine leukemia virus infections are transmitted.

118. AUSTRALIAN ANTIGEN, HEPATITIS VIRUS AND ITS RELATION TO LEUKEMIA (RABBITS)

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This is part of a broader project. A summary of this subproject is not available.

119. LEUKEMIA-ASSOCIATED CELL SURFACE ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

120. SEROLOGIC SCREENING FOR CONTAGION OF HUMAN SARCOMAS

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OBJECTIVES: (1) To gather, in a prospective fashion, sera from patients with sarcoma, their immediate and distant family members and suitable age and sex-matched controls. (2) To use such sera, particularly those from immediate family members, to search for new sarcoma-related antigens that may only be expressed in persons who have successfully mounted an immune response against the etiologic agent of sarcomas. (3) To survey the sera gathered to determine the presence of antibody to S1, S2, S3 and any newly identified sarcoma-related antigens. Such determinations will be serial for each individual followed in order to observe the frequency of seroconversion. By comparing antibody prevalence and the ratio of seroconversion to sarcoma antigens in immediate and distant family members it is intended to learn whether any of the sarcoma antigens are likely to be associated with a transmissible agent.

121. EXPRESSION OF GENES FOR TUMOR-SPECIFIC ANTIGENS

Velicer, L. F., Michigan State University, School of Veterinary Medicine, Dept. of Microbiology & Public Health, *New Administration Bldg., East Lansing, Michigan*, 48823, United States of America

The proposed research is a long-term project to purify and characterize the transformation-specific feline oncornavirus membrane antigen (FOCMA) which is on the surface of feline leukemia virus (FeLV) and feline sarcoma virus (FeSV) transformed cells and is involved in tumor immunity; and to study expression of genes for FOCMA through analysis of virion and intracellular virus-specific mRNA species and their in vivo and cell-free translation. Protein purification and characterization

will focus on physical, chemical, and immunologic properties of FOCMA using standard methods of protein chemistry and immunology to provide antisera, purified FOCMA and information needed for subsequent work on intracellular antigen synthesis, processing, and assembly, as well as on cell-free synthesis of FOCMA. The same materials will be used to develop faster and more sensitive assays for FOCMA and its antibody and to assess directly the role of the purified antigen and its monospecific antibody in immune protection and immune therapy against tumors. Virus-specific RNAs in FOCMA positive nonlymphoid cells productively and nonproductively transformed by FeLV will be analyzed by analytical cDNA probes to determine their amounts, number, size, and location in the cell. Using a preparative mercurated cDNA probe in conjunction with SH-agarose, intracellular virus-specific RNA will be purified free of cell RNA in quantity and quality sufficient for chemical analysis and for cell-free translation to determine its role as mRNA for FOCMA. Translation of intact and fragmented FeLV and FeSV virion RNA and various size intracellular viral mRNAs in mRNA dependent systems will be done to determine the coding potential of the entire genome and the translation products of each purified viral mRNA, with emphasis on determining the nature of the RNA coding for FOCMA and/or transforming gene-specific polypeptides.

122. ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGENS

Essex, M. E., Cotter, S. M., Chen, A., Harvard University, Boston Campus, School of Public Health, Dept. of Microbiology, *55 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The feline oncornavirus-associated cell membrane antigen (FOCMA) is expressed on feline sarcoma virus (FeSV) transformed nonproducer (NP) fibroblasts as a polyprotein which also contains the feline leukemia virus (FeLV) gag gene peptides p15 and p12. The same antigen is expressed on cat virus producer and NP lymphoma cells as a 65,000-70,000 dalton molecule containing only FOCMA. This antigen is not on FeLV-infected normal cells. The FOCMA molecule is an integral cell membrane protein that is apparently non-glycosylated and it lacks protein kinase activity. Little or no FOCMA activity is found in the cytoplasm or nucleus. FOCMA as expressed on FeSV-transformed NP fibroblasts shares one major cross-reactive antigenic determinant with FOCMA on lymphoid tumor cells. An additional antigenic determinant was found on the FOCMA induced by FeSV (FOCMA-S) that is not on the FOCMA found on the lymphoid tumor cells (FOCMA-L). A significant proportion of cats that were naturally exposed to FeLV also have antibodies to the FOCMA-S specific antigen.

BIBLIOGRAPHIC REFERENCES: Essex, M., Sliski, A.H., and Hardy, W.D., Jr. 1979. FOCMA: A Transformation Specific RNA Sarcoma Virus Encoded Protein. In Antiviral Mechanisms in Control of Neoplasia, P. Chandra, ed., Plenum, pp. 125-137. Grant, C.K., Ramaika, C., Madewell, R., Pickard, D.K., and Essex, M. 1979. Complement and Tumor Antibody Levels in Cats, and Changes Associated with Natural Feline Leukemia Virus Infection and Malignant Disease. Cancer Res. 39:75-81.

123. BIOCHEMICAL CHARACTERIZATION OF FOCMA

Essex, M. E., Harvard University, Cambridge Campus, School of Public Health, Dept. of Microbiology, *Cambridge, Massachusetts*, 02138, United States of America

OBJECTIVES: Conduct biological experiments to characterize feline oncornavirus cell membrane antigen (FOCMA) as a product of the feline sarcoma virus (FeSV) src gene. Conduct biochemical studies to characterize FOCMA as a product of the FeSV src gene.

124. CHARACTERIZATION OF THE FELINE ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGEN INDUCED BY FELINE SARCOMA VIRUS TRANSFORMATION

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The aim of this project is to further characterize the feline oncornavirus-associated cell membrane antigen (FOCMA) expressed on feline sarcoma virus (FeSV) transformed cells. Specifically, (1) the expression of FOCMA on nonproducer FeSV transformed cells of various species was defined by membrane immunofluorescence, (2) FOCMA was characterized biochemically to determine its size and whether there were determinants unique from the virus structural proteins, (3) hybridomas producing monoclonal antibody to FOCMA were developed in order to purify the protein and to employ the antibodies in an immunotherapy program for feline leukemia, and (4) to determine if FOCMA is a true tumor specific antigen and whether it is encoded by the virus and induced upon transformation independently of virus replication.

BIBLIOGRAPHIC REFERENCES: Essex, M., Sliski, A. H. and Hardy, W. D. Jr. FOCMA: A transformation specific RNA sarcoma virus encoded protein. In P. Chandra (ed.): Mechanism of Neoplasia. New York, Plenum Press, 1979, pp. 125-138. Essex, M., Grant, C.K., Sliski, A. H. and Hardy, W. D. Jr. Feline leukemia and immunological surveillance. In P. Chandra (ed.): Mechanism of Neoplasia. New York, Plenum Press, 1979, pp. 427-438.

125. PRODUCTION OF ANTIBODIES TO TUMOR ASSOCIATED ANTIGENS

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Tumor associated antigens (TAA) are solubilized from plasma membranes of MST (Moloney sarcoma in BN rats), LM2 (Moloney sarcoma in Lewis rats), BC3 and BC5 (sarcomas in BN rats induced by methylcholanthrene). TAA are purified on controlled-pore-glass columns, yielding fractions devoid of viral antigens, fetal antigens and histocompatibility antigens. TAA will also be prepared from the cytosol of these cells by passing the cytosol through an immunoabsorbent column of anti-TAA antibodies. Rabbits and goats are immunized with membrane TAA and cytosol TAA. Cell hybrids are formed between mouse myeloma cells and rat immune spleen cells and are cloned for monoclonal antibodies to these TAA. These monoclonal antibodies will be used to form immunoabsorbent column and to further purify TAA. The relationship between membrane TAA and cytosol TAA will be determined by pulsing with radioactive amino acid followed by cold chase. The usefulness of these homogenous TAA, anti-TAA antibodies and biochemically modified TAA (conjugated to tubercle bacilli, DNP and dodecanoic acid) as vaccines and immunotherapy will be studied.

126. THE IMMUNE RESPONSE TO VIRALLY DETERMINED TUMOR ANTIGENS

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These studies involve the interaction of humoral antibodies with lymphocytes to affect tumor cell destruction and basic mechanisms of the influence of antibodies on cell-mediated immunity. We propose to raise antibodies against monoclonal anti-M-MuSV antibodies to determine if we can induce antitumor immunity using these anti-idiotypic antibodies. Lymphocytes bind IgM complexes in the presence of Mg(plus plus) but not in the presence of Ca(plus plus). Macrophages bind IgM complexes in the presence of Ca(plus plus) but not in the presence of Mg(plus plus). In contrast, IgG binds to both independently of divalent cations. Human (Fc)5 mu chain is being radiolabeled to evaluate its binding of different subpopulations of lymphocytes. C3 receptor bearing T cells appear in mouse thymus following M-MuSV infection. We will evaluate functional capabilities of C3 receptor bearing T cells and their Ly phenotype. The cells able to lyse antibody-sensitized target cells include T cells. The cells in the thymus possessing the lowest amount of thy 1.2 were the highest in their lytic capacity against antibody sensitized target cells. We will determine the Ly phenotype of the active K cells in the thymus. In a hapten-carrier system IgM anti-carrier potentiates helper activity and IgG anti-carrier produces suppressor activity. T cells are required for the IgM dependent helper activity. These data contribute to our under-

standing of the interaction of humoral antibodies with T cell-mediated immune functions. Some T cell-mediated events can be directed by humoral antibodies. We may be able to activate patients' own T lymphocytes against their tumors using the appropriate specific antibody.

D. HERPESVIRUS-ASSOCIATED ANTIGENS

127. PRELIMINARY STUDIES FOR THE DEVELOPMENT OF AN EPSTEIN-BARR VIRUS VACCINE

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OBJECTIVE: To purify those EB virus-determined cell membrane antigens which appear to induce virus neutralizing antibodies, with a view to determining their antigenicity and suitability for use as an antiviral vaccine.

APPROACH: EB virus membrane antigens are being purified and characterized by a variety of immunochemical and immunological procedures. The purified antigens will be tested in rabbits for immunogenicity and the neutralizing power of the antibodies induced will be determined using in vitro transformation assays for EB virus infectivity. Immunogenic preparations will subsequently be inoculated into subhuman primates susceptible to EB virus to test their ability to induce protective antibodies.

128. STUDY OF EPSTEIN-BARR NUCLEAR ANTIGEN

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The specific aims of this proposal are: 1. To characterize EBNA as a protein including the elucidation of its primary amino acid sequence. 2. To study the binding of EBNA to DNA and to protein. 3. To prepare a monospecific EBNA heteroantiserum. 4. To develop radioimmunoassays for EBNA and EBNA antibody. 5. To study the possible heterogeneity of EBNA. 6. To study the functions of EBNA in their relationship to transformation of human B lymphocytes.

129. ANALYSIS OF THE EPSTEIN-BARR NUCLEAR ANTIGEN

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The transformation of human B lymphocytes by Epstein-Barr virus results in the appearance of a nuclear antigen (EBNA). Evidence indicates that nuclear antigens, which can be observed in cells transformed by many DNA tumor viruses, mediate transformation. We propose to isolate the nuclear antigen from the Raji cell line, and to examine the possibility that EBNA, as defined, is a heterogeneous protein, including the elucidation of its primary amino acid sequence. The possible heterogeneity of EBNA will be examined by immunological isolations employing a variety of antisera and the characterization of EBNA in clones exhibiting unusual nuclear fluorescence with the anticomplement immunofluorescence assay. The material and information obtained from these studies will aid in future investigations concerning the binding of EBNA to DNA and protein, and ultimately in discerning the role that EBNA plays in the transformation of human B lymphocytes.

130. A NEW CLASS OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN

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We have discovered that the Class II Epstein-Barr virus nuclear antigen (Class II EBNA), which is tightly bound to the DNA, is antigenically different from the classical EBNA which we have termed Class I EBNA and, more importantly, is related to a histone or histone-like protein which appears to be part of the EBNA complex. We now desire to purify the antigen ('protein?') and characterize its composition. We plan to compare its cell type specificity, i.e., determine whether or not it exists in EBV uninfected cells (BJAB). Eventually we wish to prepare specific antisera against this 'protein' and assess its effect on chromatin and DNA structure and template capacity (for RNA synthesis) and determine any DNA sequence specificity.

131. STUDIES OF EPSTEIN-BARR VIRUS-DETERMINED ANTIGENS

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It is the aim of this proposal to obtain various Epstein-Barr virus (EBV)-determined antigens, especially the D (diffuse) and the R (restricted) components of the EBV-induced early antigen (EA) complex in sufficient quantity and purity to determine their nature and functions, to answer questions concerning the differential antibody responses to EA-D and EA-R observed in EBV-associated diseases which cannot be resolved by presently available immunofluorescence techniques, and to prepare monospecific antisera in animals for immune electron-microscopic study of the sites and temporal aspects of antigen synthesis in productively infected cells. These aims reduce development and use of serologic techniques suitable for detection of solubilized EBV-determined antigens such as immune-adherence hemagglutination and enzyme-linked immunosorbent assays which in turn might become more widely available than the complex immunofluorescence tests for the serodiagnosis of primary EBV infections as well as Burkitt's lymphoma and nasopharyngeal carcinoma and the monitoring of tumor patients following therapy. Attempts will be made also to find an explanation for the transient emergence of heterophile antibodies in infectious mononucleosis.

BIBLIOGRAPHIC REFERENCES: Fleisher, G., Henle, W., Henle, G., Lennette, E. T., and Biggar, R. J. Primary Epstein-Barr virus infection in American infants: Clinical and serological observations. J. Inf. Dis. 139:553-558, 1979. Fleisher, G., Lennette, E. T., Henle, G., and Henle, W. Incidence of heterophil antibody responses in children with infectious mononucleosis. J. Pediatrics 94:723-728, 1979.

132. SEARCHING FOR THE EBV GENOME IN HUMAN TUMORS USING THE PAP TECHNIQUE

Sutherland, J. C., Levine, P. H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Clinical Oncology Branch, *Baltimore, Maryland,* 21201, United States of America

Because of the ubiquity and known oncogenicity of EBV, it is appropriate to determine if EBV antigen can be identified using the PAP technique in paraffin-embedded sections of a variety of human tumors. Thus far, in working with positive controls, we have identified the VCA antigen in cultured Burkitt cells which were fixed in formalin and embedded in paraffin. Thus far we've been unable to identify the EBNA antigen in similarly processed Raji cells. We are now preparing to use a freshly available monoclonal antibody to EBV utilizing both the PAP and immunofluorescent techniques on both paraffin-embedded and frozen material.

133. EPSTEIN-BARR VIRUS MEMBRANE ANTIGEN

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No summary has been provided to the Smithsonian Science Information Exchange.

134. CASE-CONTROL STUDY ON THE ASSOCIATION OF HERPESVIRUS WITH CERVICAL CANCER IN HUNGARY

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The project aims to establish herpes simplex virus-specific and cytomegalovirus-specific antigen positivity in cervical cells of women with and without cervical dysplasia. Selected antigen negative and positive cases will be followed up for years registering incidence of dysplasias and tumours in both groups. By this prospective study it is intended to identify whether anaplastic changes of the cervix uteri originate from antigen-carrying cells or not. Cervical imprints and biopsy samples are taken and coded at the Department of Oncology before sending them to the Virus Laboratory for detection of viral antigens by indirect immunofluorescence technique. It is estimated that a total of 1,000 women will be followed up.

135. HERPES TYPE 2 VIRUS AND CARCINOMA OF THE CERVIX IN NIGERIANS

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Previous serological studies in Ibadan have shown an association between the incidence of type 2 virus and carcinoma of the cervix. Further studies were undertaken to explore the role of the virus, if any, in the genesis of the malignancy by demonstrating the presence of the virus antigen in cervical smears from carcinoma of the cervix patients and its absence in smears from healthy controls, using various immunological methods. The patients were chosen from among those at the Dept. of Obstetrics and Gynecology, U.C.H., while the controls were chosen from among patients attending the family planning clinics of the hospital. Desquamated cervical cells from carcinoma patients and healthy controls were tested for the presence of viral antigens, by the direct immunofluorescence method, using FITC conjugated rabbit antiserum against herpes type 2 virus, and by the indirect immunofluorescence method, using human serum and FITC conjugated rabbit anti-human globulin. Studies are continuing, and biopsy samples from carcinoma of the cervix patients as well as from healthy controls are being collected. The samples are homogenized, and the homogenates are used as antigens for immunization of rabbits. The antibodies so produced are tested against various known antigens, both viral and non-viral, to determine the types of antigens that are present in the cervical biopsy specimens.

136. HERPESVIRUS ANTIGENS IN TRANSFORMED CELLS

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Work is in progress to develop continuous cell cultures of cells originating from cervical squamous cell carcinomas. Cells will be examined for the expression of HSV-associated antigens using immunofluorescence, immunoperoxidase, and electron-microscopic procedures. In addition, primary cell cultures and explant outgrowths of cervical fragments originating from individuals with squamous metaplasia, dysplasia and carcinomas in situ will be examined for HSV-associated antigens.

Matching sera will be tested for the presence of antibodies to HSV-1 or HSV-2 using virus neutralization or passive hemagglutination techniques. The presence and titer of antibodies to HSV will be correlated to the stage of cervical disease and to the expression of HSV-associated antigens in cervical biopsy materials and in cultured cells derived from these biopsies.

137. IMMUNODIAGNOSTIC MEASURES IN CERVICAL CANCER

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The objective of this study is to systematically assess the reliability and validity of the AG-4 Serological Test in predicting the subsequent course of cervical cancer. This tumor-specific antigen has been shown to be present in most of the patients with invasive cervical cancer or with recurrent cervical cancer, to be virtually absent in controls without cervical disease and to be present in a minority of patients with pre-neoplastic cervical disease. Patients in various stages of cervical neoplasia are being followed prsopectively in order to measure their blood levels of AG-4 before, during and after invasion.

138. MONITORING OF HERPES SIMPLEX VIRUS TUMOR-ASSOCIATED ANTIGENS IN HUMAN MALIGANT NEOPLASIAS

Tarro, G., DiGioia, M., Battista, A., Smeraglia, R., Cocchiara, R., Flaminio, G., Papa, C., Universita degli Studi di Napoli, Cotugno Hospital, Dept. of Virology, *Quagliariello, Naples, Italy*

The detection of HSV nonvirion antigens in selected tumors as expression of a repressed viral genome proves a continuing release of virus specific messages and supports an important role of the virus in the development of the tumor.

The purification of nonvirion antigen induced by HSV facilitates the detection of antibody for HSV nonvirion antigens in patients with certain cancers without absorption of their sera with HSV virion antigens. Furthermore, the preparation of specific antibody for these special antigens (nonvirion) is now relatively at hand in many laboratories for the investigation of the immune response of cancer and control populations.

PURPOSES AND AIMS: 1. To obtain purified preparations of HSV tumor-associated ('nonvirion') antigens (TAA) for use in radioimmunoassay and ELISA with cancer and control sera. 2. To monitor the presence of specific TAA antibodies in patients with different tumors of the oral and urogenital tract. 3. To correlate the clinical stage of the neoplastic disease with the presence of antibody to HSV-TAA. 4. To determine the difference in the amounts of HSV-TAA antibody between pre-operative patients and those clinically cured.

139. HERPESVIRUS CELL SURFACE ANTIGENS AND TUMORIGENICITY

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This project involves a genetic and immunological study of the interactions of herpes simplex virus type 1 (HSV-1) cell-surface antigens induced in infected and transformed hamster embryo fibroblasts with the immune system. Special attention is paid to (1) the role of these antigens in complement-dependent and cell-mediated immune cytolysis and (2) the role of virus-induced Fc receptors in blocking immune cytolysis. Evidence has been provided that virus-induced Fc receptors on infected and transformed cells can combine with Fc fragments, agg-IgG and immune complexes to block cell-mediated and complement-dependent immune cytolysis. Fc receptors in vivo may combine with blocking antibody or immune complexes, thus protecting tumor cells against the host's immune system. Genetic, biochemical and immunological techniques are employed to isolate, identify and characterize the virus-specified antigens. Hamster embryo cells infected with wild-type HSV-1 (KOS) and its temperature-sensitive mutants are analyzed for their spectrum of cell-surface antigens. Some mutant-infected cells vary in their surface antigen content, including Fc receptors, under nonpermissive conditions. Surface antigens radioiodinated by the lactoperoxidase technique are isolated by tandem analytical polyacrylamide gel electrophoresis and isoelectric focusing. In situ immune complexing procedures are performed in polyacrylamide gels using 125I-labeled specific antibody and Fc fragments to identify HSV antigens and Fc receptors.

140. A MORPHOLOGIC INVESTIGATION OF VIRUS INDUCED MEMBRANE RECEPTORS

Triche, T. J., Costa, J. C., Rabson, A. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Ultrastructural Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Herpes simplex virus infected cells develop a receptor capable of binding the Fc portion of IgG at the same time viral membrane antigens appear; it is not known whether this Fc receptor is a host cell or viral protein, though the latter is more likely. Studies utilizing hemocyanin-tagged IgG to label the Fc receptor and ferritin-tagged anti-HSV antigens on virally infected cells will determine whether the Fc receptor co-labels with antibodies to HSV antigens.

141. IMMUNITY OF SUSCEPTIBLE AND RESISTANT CHICKENS VACCINATED WITH MAREK'S DISEASE TUMOR CELL ANTIGENS

Sevoian, M., University of Massachusetts, Amherst Campus, Agricultural Experiment Station, Dept. of Veterinary & Animal Sciences, *Amherst, Massachusetts*, 01002, United States of America

OBJECTIVES: Characterize a non-productive cell-line (JMV-1) as to growth, morphology, antigenic properties and lethality. Investigate tumor cell resistance in terms of the cell-mediated system. Determine the immunological effect of tumor-cell antigens in the vaccination of chickens genetically susceptible and resistant to Marek's disease (MD).

APPROACH: It is important that investigations in tumor immunity begin now utilizing non-pathogenic tumor antigens in vaccine development for prevention. Furthermore, it is possible

that antiserum to tumor antigens may some day be used in tumor immunotherapy of animals. The ground work for developing these potentials currently exists at this institution.

142. MAREK'S DISEASE HERPES VIRUS ANTIGENS

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The overall objective of this project is to identify and characterize important antigens in the Marek's disease herpesvirus (MDHV) system and to determine immunological relationships that exist, especially as related to tumor formation and its prevention through a successful vaccine. One specific aim is to further purify, characterize and compare the soluble common extracellular A antigen (MDHV-A), intracellular B antigen (MDHV-B) and possible other antigens produced by cells infected either with MDHV or the vaccine virus, herpesvirus of turkeys (HVT), and to prepare high titered monospecific antisera against them. Previous work with the MDHV-A and -B led to their identification as glycoproteins and their 200-fold purification with 25% recovery. Purified antigens and their monospecific antisera will be used to determine their relationship, if any, to each other, virion structural proteins, neutralizing antibody, interferon, and delayed hypersensitivity in infected birds. Plasma membranes from productively infected cells will be radiolabeled, purified, solubilized with detergents and analyzed by immune methods and SDS-PAGE for virus-specific proteins and antigens in order to determine the nature of membrane antigen (MA) which is currently defined by immunofluorescence. Special emphasis will be placed on possible relationships between soluble MDHV-A and MDHV-B or other antigens and the MA or other membrane associated antigens. Plasma membranes from a lymphoblastoid cell line will be analyzed in a similar manner to determine the nature of the Marek's disease associated tumor surface antigen (MATSA) which is also defined by immunofluorescence and is unrelated to the antigens described above. Radioiodination, immune co-precipitation and SDS-PAGE analysis are being used to identify MATSA. Plasma membranes from both types of cells, their solubilized components, and soluble counterparts will be purified and assayed for their ability to immunize and protect chickens against Marek's disease to determine the relative importance of virus-specific and tumor cell-specific antigens in protection.

BIBLIOGRAPHIC REFERENCES: Velicer, L.F., D.R. Yager, and J.L. Clark. 1978. Marek's disease herpesviruses. III. Purification and characterization of Marek's disease B antigen. J. Virol. 27:205-217.

143. EBV AND HERPESVIRUS SAIMIRI INDUCED MEMBRANE ANTIGENS

Pearson, G. R., Qualtiere, L. F., Mayo Foundation, Dept. of Microbiology, *200 1st St. S.W., Rochester, Minnesota,* 55901, United States of America

Herpesviruses have been implicated as etiological agents in some forms of animal and human cancers. This has raised the possibility of prevention of these cancers through the use of viral vaccines. Because of the potential danger of using attenuated or inactivated herpesviruses containing intact viral genetic information for the induction of immunity in human populations, it would appear to be more realistic to consider using purified viral proteins free of viral DNA for inducing a protective immune response against the oncogenic virus or the virus-induced transformed cell. This requires the purification and characterization of the appropriate viral antigens. In some of the herpesvirus systems, including herpes simplex virus and Epstein-Barr virus, these antigens appear to be expressed in the membranes of infected cells. The major objectives of this research proposal are: 1) to continue the isolation and purification of membrane antigens induced by EBV and herpesvirus saimiri, an oncogenic nonhuman primate virus; 2) to produce monospecific antisera against these components for utilization in studies directed at defining the relationship of MA to other virus-associated antigens; 3) to identify the EBV antigens expressed in infected or transformed cells that serve as targets for antibody dependent cellular cytotoxicity (ADCC); and 4) to assess the potential use of purified MA components as possible subviral vaccines for prevention of tumor induction by these 2 viruses in owl monkeys. Membrane fragments prepared by solubilization of virus-infected cells with Triton X-100 will serve as the main source of MA. Radioimmune precipitation will serve as the main assay for identifying virally induced glycoproteins. Purification schemes will employ primarily immunoaffinity and lectin chromatography and preparative polyacrylamide gel electrophoresis.

144. DIFFERENTIATION AND VIRAL ANTIGENS ON PRIMATE B LYMPHOID CELL LINES

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The objective of this research is to elucidate the membrane arrangements and surface interactions occurring between lymphotropic herpesvirus structures and B lymphocyte differentiation antigens on primate lymphoblastoid cell lines. A major hypothesis to be tested is whether specific interactions between viral and lymphocyte surface gene products occur which are related to the differentiated state of the cell or whether or not the cell is latently infected or entering the virus lytic cycle. Initial efforts will center on developing and characterizing monoclonal antibodies specific for B lymphocyte differentiation antigens and selected virus-coded membrane antigens. Using these specific probes and methods designed to detect associations between membrane gene products, viral-differentiation antigen arrangements or nonproducer and virus producer cell lines will be determined. Changes in the composite surface phenotype of cells induced to produce viral antigens will be monitored. Family studies and interspecies testing with monoclonal antisera will be undertaken in order to define polymorphic determinants on primate B cells. These markers will be used to examine the relationship between B cell surface phenotype and susceptibility to virus-induced transformation.

145. CYTOPLASMIC ANTIGENS OF HERPESVIRUS IN CANCER CELLS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

E. SIMIAN VIRUS 40 ANTIGENS

146. CHARACTERIZATION OF TUMOR ANTIGENS PRESENT IN SV40-TRANSFORMED MOUSE CELLS

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BALB/c 3T3 cells transformed by SV40 express, in addition to normal SV40 T-Ags, a variety of other proteins which specifically immunoprecipitate with SV40 anti-T serum. Preliminary work on the relationship of these proteins to normal SV40 T-Ags had shown that they consist of two groups. One group is closely related to normal SV40 T-Ags while the other has little or no relationship to SV40 T-Ags and is referred to as non-viral T antigen (NVT), e.g., 53K.

Several SV40-transformed cell lines have since been screened for the presence of abnormal T-Ags and we find: 1. elongated forms of large T (super T); 2. forms of large T with mobilities similar to but distinct from normal sized large-T; 3. shortened forms of large T (truncated large T MW 15-50K).

We have further characterized these proteins in some detail by two-dimensional tryptic fingerprint analysis.

All of the abnormal proteins, like normal SV40 large-T, are phosphorylated and all have the usual N terminal peptide N.Ac.Met.Asp.Lys. All the truncated large T's examined have the peptides shared by large-T and small-t; i.e., the N terminal region. In some cases no additional large-T unique peptides are detected. Super-T has the same pattern of methionine and cysteine peptides as normal large-T. No additional peptides are found; however, some peptides may be present in multiple copies. Thus there may be a duplication of part of the large-T molecule. Tentative mapping of these duplicated peptides suggests that the duplicated peptides are within 35-40K of the carboxy terminus of the large-T coding frame.

We have studied the abnormal T-Ags by cell-free translation of mRNA from these cells selected by hybridization with SV40 DNA. The pattern of proteins synthesized in vitro is

virtually identical to that found by in vivo labelling. This suggests that the abnormal T-Ags are probably primary translational products and do not result from extensive post-translational modifications.

Currently we are sizing the mRNA of these proteins. This may enable us to correlate the structure of the integrated viral DNA and mRNAs with the proteins they encode.

147. PURIFICATION AND INVESTIGATION OF THE BIOLOGICAL PROPERTIES OF SV40 SMALL-T MRNA

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To investigate the function of individual SV40 tumor antigens, we have purified the mRNAs for SV40 small-t and large-T and are attempting to introduce the purified mRNAs into living cells. In this way it may be possible to observe the biological effects of the SV40 proteins following their synthesis in injected mammalian cells. For the first experiments, however, we have used purified small-t mRNA and injected it into Xenopus oocytes.

A fragment of SV40 DNA containing the sequences between 0.59 and 0.54 map units (which encode the unique portion of SV40 small-t) was isolated using the restriction enzymes Hae III and Alu I. The purified fragment was cloned in the plasmid vector pBR322 (under GMAG containment category 2). The linearized plasmid DNA was covalently attached to cellulose and the resin used to isolate small-t mRNA by preparative scale hybridization.

The purity of the mRNA was checked by translation in a cell-free system. This showed that the purified fraction contained mRNA highly active in the synthesis of small-t with virtually no contamination with mRNA coding for SV40 large-T.

To test the biological activity of the purified mRNA it was injected into Xenopus oocytes. Pure small-t mRNA directed the synthesis of small-t but not of large-T, whereas total SV40 mRNA directed the synthesis of both large-T and small-t. This result suggests that small-t mRNA is not spliced to give large-T mRNA in the cytoplasm of living cells.

Further experiments in which we inject mRNA into the oocyte nucleus and into mammalian cells are in progress.

148. CHARACTERIZATION OF THE C-TERMINAL REGION OF SV40 T-ANTIGEN

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The large-T antigen polypeptides produced in cells infected with SV40 deletion mutants dl 1263 and dl 1265 have been characterized by analysis of their tryptic peptides with particular emphasis on the C-terminal region. The T-antigen of the wild type SV-S produces a proline-rich C-terminal peptide which is lacking in digests of dl i265 T-antigen. This defines the location of this peptide at the C-terminus of the protein and confirms the reading frame used in the translation of this part of the genome. The altered C-terminus of dl 1265 T-antigen is cysteine rich and this affects the electrophoretic properties of polypeptide markedly. The proline-rich terminal peptide is still present in digests of dl 1263 large-T antigen showing that translation continues after the deletion and terminates normally. Because of the deletion in dl 1263 the molecular weight of its large-T antigen should be slightly less than that of SV-S. In fact, its apparent molecular weight is considerably less than that of SV-S. Since dl 1263 T-antigen retains the proline-rich C-terminal tryptic peptide we can be certain that this larger than expected reduction in apparent molecular weight is not due to loss of the C-terminal region by premature termination of translation or by proteolytic degradation of the completed polypeptide.

Both dl 1263 and dl 1265 transform mouse cells and the major forms of T-antigen produced in the transformed cells were indistinguishable from those produced in infected cells. They also appeared to associate with the host 53K to form a complex in the same way as the SV-S large-T antigen (Lane and Crawford, Nature, 1979, 278, 261).

149. CHARACTERIZATION OF THE MIDDLE T ANTIGENS OF SV40

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There now appear to be multiple SV40 tumor antigens - large T, small T, and one or more 'middle' T antigens. A role in the virus-induced neoplastic transformation process has been assigned to large and small T. To date, we know little that would suggest a role for SV40 middle T antigens, but they do exist for polyoma virus, as well, and at least one has been found in association with the cell surface in that system. In the case of SV40, they appear, thus far, to be present in transformed cells and from preliminary qualitative peptide map studies do not appear to be simply cleavage products of large T. This information does suggest that these proteins are, to a limited degree, subsets of large and small T and in part unique. They might therefore result from splicing (in the messengers) of regions of the T antigen coding segments with either host cell sequences or with other presumably early region segments which are large and/or small T antigen coding segments but are now read in another frame to give new polypeptide sequences.

I will attempt to purify these polypeptides in denatured form from one or more SV40 transformants by selective immunoprecipitation and preparative SDS gel electrophoresis. The isolated polypeptides - once obtained in pure form - will be subjected to one or more methods of cleavage. I will seek a cleavage method which results in a small number of large peptides which can be readily separated into pure species. Each peptide would then be sized and subjected to N- and C-terminal analysis, N- and C-terminal sequence analysis, and amino acid analysis. By correlating this information with the known sequence of SV40 DNA, it should be possible to derive useful information on the location and organization of the coding regions for these proteins.

150. ISOLATION AND FUNCTION OF SMALL SV40 T ANTIGEN

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No summary has been provided to the Smithsonian Science Information Exchange.

151. STRUCTURAL ANALYSIS OF THE SV40 TUMOR ANTIGEN

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The purpose of the proposed research is to compare structurally several proteins observed in immunoprecipitates of SV40 infected cells. The proteins include two known products of the SV40 early genes, proteins with molecular weights of 94,000 and 17,000 daltons. Two additional proteins with molecular weights of 56,000 and 32,000 daltons are also present in cells infected with wild-type virus, but are absent from immunoprecipitates of cells infected with certain non-transforming deletion mutants. The possibility that the 56K and 32K proteins are only observed when complexed to the 17K viral gene product is being explored.

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152. STRUCTURAL ANALYSIS OF THE SV40 TUMOR ANTIGEN

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This is part of a broader project. A summary of this subproject is not available.

153. CHARACTERIZATION OF SV40-SPECIFIC MEMBRANE ANTIGENS

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The long-term objective is to characterize structure-function relationships of the two simian virus 40 (SV40)-specific membrane antigens defined as surface (S) and perinuclear (U) antigen. In these studies, SV40-transformed lymphocytes (GD248) will be used initially; later we will also employ a human SV40-transformed lymphoid cell line. The projects planned can be subdivided into three major categories: 1. Quantitative distribution of the two antigens in different subcellular membranes. 2. Spatial distribution and neighborhood relationships of these antigens within the membrane. 3. Their chemical characterization in different membranes to assess the mechanisms of host cell modification of one original SV40 gene product. Working with lymphoid cells neoplastically transformed with SV40 we plan: (a) To establish a human lymphoid cell line derived from T- or B-cells transformed in vitro with SV40; (b) to prove whether both S- and U-antigens are expressed on the cell surface; (c) to define their quantitative distribution in the outer envelope of the nuclear membrane (domain of U-antigen), in membranes of the endoplasmic reticulum and in the plasma membrane (domain of S-antigen); (d) to analyze the spatial distribution of S- and U-antigens across the membrane using controlled proteolysis and labeling techniques from both sides of the membrane; (e) to specifically investigate the neighborhood relationships between SV40 and HL-A antigens in plasma membranes of human lymphoid cells using (1) hydrophilic and hydrophobic photoactivated reversible cross-linking agents followed by (2) immunochemical analyses of cross-linked membrane proteins/glycoproteins with sera specific for HL-A, S- and U-antigens; (f) to isolate SV40-induced S- and U-antigens by preparative isoelectric focusing on the basis of their extreme isoelectric points, pI 4.5 and pI 4.7 respectively; (g) to chemically characterize the protein and carbohydrate moieties of the two groups of antigens using (1) N-terminal amino acid analyses, (2) peptide/glycopeptide mapping, (3) amino acid analyses of peptides (glycopeptides), and (4) bulk and sequential analyses of the oligosaccharide residues.

154. CELL SURFACE CHANGES IN TRANSFORMED MOUSE CELL LINES

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OBJECTIVES: To study surface and other changes in transformed cells and the molecular mechanisms responsible for antigenic changes and/or the transformation to malignancy, either spontaneously or as induced by SV40 virus. To gain a better insight into the biological activity and function of new proteins and antigens which appear after transformation. To develop better methods to isolate and purify cell surface membranes, new cellular antigens, and antigenic components such as SV40 virus specific T antigens, surface antigens and tumor specific transplantation antigens; and to study their interrelationships.

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155. CELL SURFACE CHANGES IN SPONTANEOUSLY OR SV40 TRANSFORMED MOUSE CELL LINES

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The purpose of this project is to study the cell surface membrane -how it may change in transformation to malignancy either by spontaneous induction or as induced by SV40 virus; to study the nature of tumor antigens associated with cells transformed by either SV40, chemicals, or spontaneous induction; and the relation of these antigens to gene expression and host immune mechanisms. Research is carried out on: 1) Changes in glycoconjugates, including glycoproteins and sulfated glycosaminoglycans such as heparan sulfate, and their correlation with cell growth properties in tissue culture and in vivo, in the syngeneic and in the nude mouse; 2) controls of expression and methods of purification of SV40 specific T antigens, surface antigens and tumor specific transplantation antigens (TSTA); 3) the relationship between SV40 T, surface and TSTA antigens, and their relationship to SV40 A gene expression; 4) a 56,000 MW SV40 induced but cell DNA encoded protein which is also present in embryonal carcinoma cells and in certain embryo cells; 5) study of 'spontaneous' transformation of cells in culture and of the balance between such tumorigenicity and the SV40 TSTA in families of clonal cells with known genealogy.

BIBLIOGRAPHIC REFERENCES: Simmons, D. T., Martin, M. A., Mora, P. T., and Chang, C.: Relationship among TAU antigens isolated from various lines of simian virus 40-transformed cells. J. Virol., 1980, in press. Chandrasekaran, K., Winterbourne, D. J., Luborsky, S. W., and Mora, P. T.: Surface proteins of simian virus 40 transformed cells. Virology, 1980, in press.

156. MEMBRANE ANTIGEN ORGANIZATION IN TUMOR IMMUNITY

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The lymphocytes from animals immune to syngeneic simian virus 40 (SV40)-induced tumors recognize both virus-induced and histocompatibility-2 (H-2) antigens on SV40 transformed target cells. Evidence presented here suggests that T cells bind to a complex between H-2 and the viral antigen on the tumor cell, and formation of this complex is necessary, if not sufficient, for induction of cytolytic T cells and for in vitro tumor destruction by T cells. In the work proposed, we will investigate the formation of antigen complexes in the tumor cell plasma membrane and the relationship between complex formation and immune T cell recognition.

Co-capping of tumor-associated antigens with mouse H-2 antigens, reported by this and other laboratories, also indicates physical associations among plasma membrane molecules. We have recently found that antigens which co-cap can also be extracted from the membrane as specific complexes. Both the murine leukemia virus gp71 and a carcinofetal antigen, Ag I, are associated with the H-2 antigens following solubilization of L cell membranes with low concentrations of non-ionic detergents. We will study the requirements for formation of these complexes and the relationship between complex formation and endogenous virus production.

We will establish a model system for T cell recognition of foreign proteins expressed on syngeneic cells. This model will allow quantitative evaluation of immune recognition and manipulation of the immune response to cell surface antigens.

157. CHARACTERIZATION AND THERMOSENSITIVITY OF SV40 MEMBRANE ANTIGEN

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This is part of a broader project. A summary of this subproject is not available.

158. SV40 T ANTIGEN - ITS ENZYMATIC ACTIVITIES AND ROLE IN DNA SYNTHESIS

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We propose to study the role of SV40 large T antigen in the stimulation and control of cellular DNA synthesis. We will purify the D2 hybrid protein, a protein that is specified by the adenovirus-SV40 hybrid Ad2(plus)D2, and that is functionally identical (to the extent that it has been studied) to large T antigen. This protein binds to the origin of the SV40 genome, stimulates cellular DNA synthesis and is associated with protein kinase and ATPase activity.

Among the major questions about the D2 hybrid protein are whether the 2 enzymatic activities are specific for the infected cell and whether they are intrinsic to the protein. We will improve the purification procedure and then test to determine if the activities are still associated with the protein. Purity will be determined by end-group analysis, the presence of a single band in isoelectric focusing and in several different electrophoresis methods, and quantitative immunoprecipitation. To determine if the enzyme activities are unique, we will examine temperature inactivation, Km's for various substrates and other biochemical characteristics and compare them with those of known kinases and ATPases. We will also search for kinase activity and specific phosphoproteins in subcellular fractions from uninfected and lytically infected CV-1 cells. These fractions will include transcriptional intermediates and replication complexes. We will also search for specifically phosphorylated proteins in transcriptionally active and inactive chromatin, nonhistone chromosomal proteins, and nuclear matrix proteins.

Using the DNA binding assays with the S2 hybrid protein, we will determine the affinity of cellular DNA for it. If it binds, we will examine whether the bound DNA sequences are specific, hypothesizing that the specific sequences contain the origins of replication. The ability of the protein to stimulate DNA synthesis in resting cells will be tested in vitro in a lysed cell system and in vivo by microinjection using red cell ghosts. This will determine whether stimulation is direct (protein interacts with replicating DNA) or indirect (protein starts a cascade leading to replication). The newly synthesized DNA will be examined by fiber autoradiography to learn if initiation or chain elongation or both is stimulated. (Text Abridged.)

159. THE QUANTITATION OF SV40 LARGE-T AND SMALL-T ANTIGEN SYNTHESIS DURING LYTIC INFECTION

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Studies of SV40 tumor antigens rely heavily upon immunological techniques for the isolation and quantitation of the relevant polypeptides. The coding regions for the large-T and small-t overlap and the polypeptides share antigenic determinants (Lane and Robbins, Virology, 1978, 87, 182) so that they may compete for antibody directed against these determinants. In immunoprecipitation it was found that ten- to thirty-fold more antiserum was required for optimum precipitation of small-t than was needed for large-T. This effect can easily lead to underestimation of small-t or even failure to detect small amounts of small-t and related polypeptides. Using adequate amounts of antiserum for immunoprecipitation it was found that the amount of synthesis of small-t in SV40-infected cells was substantial. The ratio of small-t to large-T synthesis increased late in infection to give more than equimolar synthesis of small-t. The messenger RNAs for small-t and large-T are probably derived from the same initial transcript by alternative splicing pathways. One possible explanation for the change in ratio of small-t to large-T synthesis could then be a change in the preference for these two alternative splicing pathways.

160. CHARACTERIZATION OF A HIGH MOLECULAR WEIGHT COMPLEX CONTAINING SV40 LARGE-T ANTIGEN AND A HOST 53K PHOSPHOPROTEIN

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The sedimentation properties of SV40 large-T antigen extracted from several types of cell have been studied. In detergent extracts of SV40-transformed mouse, rat and hamster lines, large-T appears to exist in two major forms, a free form sedimenting at 5S and a high molecular weight form co-sedimenting with a host phosphoprotein at 23S in the rat and mouse extracts, and 16S in the hamster extracts. The mouse host phosphoprotein has a subunit molecular weight of 53,000, and it has been shown by immunological studies that it is bound to large-T (see Report 73). This association is non-covalent since the two proteins can be dissociated by SDS or high concentrations of urea. However, the interaction of subunits is not affected by high salt (4M NaCl), or EDTA, 0.5 percent NP40 or 2M urea.

The form of large-T that binds to the 53K host phosphoprotein appears to be more highly phosphorylated than the free form. This may be due to different rates of turnover of the phosphate groups rather than different phosphorylation sites.

161. CHARACTERIZATION OF THE COMPLEX BETWEEN SV40 LARGE-T ANTIGEN AND THE 53K HOST PROTEIN IN TRANSFORMED MOUSE CELLS

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The existence of a complex between SV40 large-T and the 53K host protein in transformed mouse cells was first suggested (ICRF Scientific Report 1978 - report 54) to account for the coordinate precipitation of the 53K protein together with large-T by a variety of antisera, including those spcific for large-T, or even for a small region of this polypeptide. This result has now been confirmed and extended in several ways. Coordinate precipitation of large-T and small-t by antisera specific for large-T, a consequence of shared antigenic determinants, has already been described. This is quite different from the coordinator precipitation of large-T and the 53K protein, since antiserum against denatured large-T has no detectable activity against the denatured 53K in the direct binding radioimmunoassay. The fact that the 53K protein is coded by the host is shown most clearly by its presence in mouse cells which are not transformed or are transformed by a virus other than SV40 (see Report 58).

In spite of its being a mouse protein it can nevertheless be antigenic in mice since some anti-T sera from tumor-bearing mice do have activity against the isolated 53K polypeptide. Its antigenicity may be a consequence of being presented to the immune system in the form of a complex with large-T.

162. TEMPERATURE-SENSITIVE INDUCTION AND ASSOCIATION OF A LARGE 53K PHOSPHOPROTEIN WITH LARGE-T ON 3T3 CELLS INFECTED WITH SV40 TSA30

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Infection of 3T3 cells with wild type SV40 (a non-permissive system of infection) results in induction of a host 53K phosphoprotein that binds to large-T (see Report 71). Infection with tsA30 at the non-permissive temperature does not result in induction of this protein although large-T and small-t are expressed. The presence of the 53K protein was tested using anti-53K serum. 3T3 cells transformed by ts-A30 express the 53K protein when maintained at the permissive temperature. Pulse chase experiments indicate that the 53K-large-T complex in these cells dissociates more rapidily at the non-permissive temperature (t1/2,4h) than the permissive temperature (t1/2, 24h). Most of the large-T synthesized following a shift-up to the non-permissive temperature fails to associate with the cellular 53K protein, although normal amounts of 53K are synthesized during this period.

163. SV40 TUMOR ANTIGEN IN SV40 NUCLEOPROTEIN COMPLEXES

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The aim of this project is to characterize the SV40 large tumor (T) antigen found in association with the SV40 nucleoprotein complexes extracted from SV40-infected cells (Mann, K. and Hunter, T., J. Virol. 29: 232 (1979)). It seems likely that this is a functional association involved in the initiation of SV40 DNA synthesis since SV40 T antigen is not detectable in nucleoprotein complexes from SV40 tsA-infected cells incubated at the nonpermissive temperature. We will, therefore, characterize the T antigen associated with the nucleoprotein complexes with respect to its chemical composition, its specific site of binding on the SV40 DNA, its relative association with SV40 (RI) DNA-containing complexes versus SV40 (I) DNA-containing complexes, the effect of salts and divalent cations on its association with the nucleoprotein complexes, its heat lability and ability to undergo renaturation, its ability to bind to nucleoprotein complexes in vitro, and its enzymatic activity. We will specifically examine modification (acetylation, glycosylation,

and phosphorylation) of the nucleoprotein complex T antigen to see if it differs from the modification found in other populations of T antigen molecules, in particular in the T antigen molecules not associated with the nucleoprotein complexes. It is possible that modification influences the ability of large T antigen to bind to SV40 DNA. We will attempt to identify the enzymatic activity of the nucleoprotein complexes associated with and those not associated with the SV40 large T antigen. The enzymatic activity of large T antigen associated with the complexes may differ from the enzymatic activity of the T antigen molecules not bound to the DNA in the nucleoprotein complexes. With these studies, we hope to further our understanding of the biological role of SV40 large T antigen in the initiation of SV40 DNA synthesis and in cell transformation.

164. SV40 T ANTIGEN BINDING SITES IN CELLULAR DNA

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The SV40 large T antigen is a DNA binding phosphoprotein. It may also be closely associated with a protein kinase activity and as such has the capacity to phosphorylate itself. The function of this antigen is necessary for SV40 induced neoplastic transformation, the initiation of SV40 DNA replication, and the control of early viral transcription. Large T purified from SV80 cells, an SV40-transformed line, binds preferentially to certain sites on the SV40 genome. I would first like to investigate whether the state of T phosphorylation affects these DNA binding properties. Specifically, I should like to determine whether the state of large T antigen phosphorylation affects both its quantitative ability to bind to various DNAs as measured by the nitrocellulose filter binding technique and also whether binding to certain specific sites on the SV40 genome is detected by nuclease protection experiments.

Secondly, there is immunofluorescent banding evidence which suggests that T antigen associates with mammalian chromosomes in a site specific manner. To pursue this possibility further, I wish to ascertain, directly, whether preferential binding sites for SV40 large T antigen exist in cellular DNA and, if so, to determine the nucleotide sequences of one or more of these loci. To answer these questions, I shall use a cloned 'library' of shotgun mouse cellular DNA as well as certain well-characterized cloned mouse genes. Using various techniques involving protection of selected sites from digestion by a specific nuclease, I shall attempt to identify and characterize any specific T antigen binding sequences which may exist.

165. NUCLEIC ACID-PROTEIN INTERACTIONS IN THE SV40 T ANTIGEN

Rubin, H., Sidney Farber Cancer Center Inc., Dept. of Medicine, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

I propose to investigate certain chemical properties of a primary early SV40 gene product - 'large' T antigen - and its binding to the SV40 genome. My goal will be to identify specific DNA sequences of specific 'large' T antigen binding sites on the SV40 chromosome. Two major approaches will be tried - protection of unique 5'-labeled DNA fragments against exonuclease III digestion and protection of specific residues against modification with dimethylsulfate. I wish to gain further insights into the chemical basis for the specific interactions of this protein and specific SV40 genomic loci.

166. BIOCHEMICAL PROPERTIES OF THE SV40 T ANTIGENS

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The early or transforming region of simian virus 40 encodes the T Ags (tumor antigens) and induces TSTA (the tumor specific transplantation antigen). The primary objectives of this research are threefold: 1) To study by physical methods and affinity columns key reactions of large T Ag (self-association, nucleotide and DNA binding, and interaction with the new 48K-55K phosphoprotein species) which are important to understanding its probable enzymatic activity in initiating DNA synthesis; (2) to characterize the new 48K-55K phosphoprotein species (isolated in my laboratory) by a) mRNA isolation and in vitro translation and b) preparation of specific antisera to determine whether they are either 1) virus coded or 2) host coded, but virus-induced or modified; and 3) to characterize the new putative cell-surface (PCS) glycoprotein species recently isolated in my laboratory, which crossreacts with large T Ag by comparison of its tryptic fingerprint with large T Ag. In addition we will determine by immunization with the isolated PCS whether it is related to TSTA, the viral antigen mediating tumor rejection. Unique to this work has been our use of antisera to pure large T Ag to isolate new species which may be the postulated viral products such as TSTA.

BIBLIOGRAPHIC REFERENCES: Carroll, R.B., Goldfine, S.M., and Melero, J.A. Antiserum to Polyacrylamide Gel-Purified Simian Virus 40 T Antigen. Virology 87: 194 (1978).

167. FUNCTION AND EXPRESSION OF SV40 VIRAL TUMOR ANTIGENS

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SV40 T antigen is involved in malignant cell transformation and viral replication. According to biological and biochemical evidence this viral gene product accomplishes its potent effects via its interactions with cellular and viral DNA. The properties of T antigen will be examined by analysis of its post-translational modifications including phosphorylation and glycosylation. The relationship of these processes to the specific DNA affinities and oligomerization of T antigen in permissive and transformed cells will be investigated. We will attempt to isolate cellular DNA sequences in transformed cells for which active T antigen has specific affinity.

Various aspects of T antigen expression will be analyzed. mRNA known to code for the T antigens will be used to study processes such as splicing and other post-transcriptional modifications. We will investigate differences in T antigen mRNA isolated from lytically infected and transformed cells in terms of these modifications and also with regard to intracellular localization and cytoskeleton. Host regulation of T antigen mRNA expression will also be studied in revertants isolated from SV40 transformants.

168. STUDIES ON THE SV40 T-ANTIGEN BINDING SITE

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The central aim of this research is to decipher how T-antigen binds specifically to three sites on the SV40 chromosome. The approach involves the following: (1) Chemical and enzymatic synthesis of the tight-binding T-antigen site (site I). (2) Site specific insertion of purine and pyrimidine analogs into this binding site. (3) Kinetic measurements of the stability of T-antigen bound to this DNA (modified and unmodified). The approach should reveal functional groups on base pairs that are recognized by T-antigen. These syntheses and binding studies will then be extended to include sites II and III and the DNA regions containing the replication origin and the early mRNA promoter. These results will be useful for deciphering the biological mechanisms associated with SV40 replication, early mRNA transcription, and maintenance of the transformed state.

169. BIOCHEMICAL STUDIES ON T ANTIGEN AND TRANSFORMED CELLS

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Simian virus tumor (T) antigen will be compared to the adeno-SV40 hybrid T antigen with respect to single and double strand DNA binding, site specificity and catalytic activities. The poly dT stimulated ATPase activity of T antigen will be defined in terms of the minimum size of the poly dT molecule required for optimum activation of the ATPase activity. The high affinity binding of poly dT to the T antigen will also be measured. The relation of the ATPase activity of T antigen to its role in the initiation of DNA synthesis will be examined. Attempts will be made to assay 'in vitro' initiation of SV40 viral DNA in the presence of purified T antigen, host polymerases and other factors required for eucaryotic DNA synthesis. Attempts will be made to convert transformed cells to normal cells by competing

T antigen off cellular DNA and onto poly dT or other high affinity binding single strand DNA species.

170. STUDIES ON ROUS SARCOMA VIRUS AND SIMIAN VIRUS 40

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In studies of SV40 early antigens, Ad2(plus) ND2 induced in infected cells two SV40 specific polypeptides of 42,000 and 56,000 molecular weight; Ad2(plus) ND1 induced a 28,000 molecular weight peptide. The Ad2(plus) ND1 segment is found to be sufficient to induce the SV40-specific tumor antigen in mice.

With 2-dimensional gel electrophoresis a 4th AAV structural protein (VP) was detected. Each of the four VP's (m.w. 86.5, 72.5 61.5, 60.5) contains multiple isoelectric species. While VP 1, 2 and 3 share many tryptic peptides, at least, VP 1 and 3 each contain unique tryptic peptides. Virion proteins as well as cell free translated proteins are presently being analyzed to test whether the VP's are overlapping or whether that frame shift also plays a part apparently as a method of conserving DNA by this virus.

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171. STRUCTURE AND FUNCTION OF SV40 NON-VIRION PROTEINS

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We are engaged in a coordinated study of the structure and function of the SV40 large T antigen. In progress are experiments designed to assess 1) the role of the two enzymatic activities associated with SV40 T antigen - ATP'ase and protein kinase; 2) the nature of non-origin region SV40 DNA binding sites; 3) the role of large T in the induction of the 55K cellular Tau protein; 4) the structure of the carboxy terminus of this protein isolated from both transformed and lytically infected cells and 5) the sites of T antigen binding in cellular DNA.

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172. BIOLOGICAL PROPERTIES OF SV40 EARLY PROTEINS

Butel, J. S., Noonan, C. A., Baylor College of Medicine, School of Medicine, Dept. of Virology & Epidemiology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The goal of this project is to elucidate and characterize biological properties of SV40 early proteins. The antigenic relatedness of SV40 large T, small t, U, and S antigens was established using antiserum directed against purified, denatured T polypeptide. The presence of large T-ag in the plasma membrane of SV40-infected and transformed cells was established. Transport-defective mutants of SV40-adenovirus 7 hybrid virus (PARA) were found to exhibit a dominant effect which inhibited nuclear migration of wild-type SV40 T-ag.

173. ORIGIN AND FUNCTION OF SV40-SPECIFIC TAU ANTIGENS

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We have been investigating the properties of a group of proteins called Tau antigens that can be isolated from various lines of SV40-transformed cells by immunoprecipitation with serum obtained from hamsters bearing SV40-induced tumors (anti-T serum). Work in several laboratories has shown that these proteins can be isolated from mouse embryonal carcinoma cells and from cells transformed by various retroviruses, papovaviruses, and chemicals. They have not been detected in significant amounts in normal, untransformed cells or in tumor antigen-negative revertant cell lines derived from SV40-transformed mouse or rat cells.

We have determined the relationship between Tau antigens isolated from various lines of SV40-transformed cells by comparing their methionine-containing tryptic peptides by two-dimensional fingerprinting. All Tau antigens studied so far appear to share some common peptides. Those isolated from closely related species (i.e., mouse and rat) show the greatest similarity. Within a certain species (mouse, hamster), the Tau antigens are very closely related or perhaps identical.

Recently, we have detected Tau antigen in monkey cells infected with SV40. This protein is closely related to the Tau antigen isolated from a line of human cells transformed by SV40.

Finally, we have compared the Tau antigens isolated from hamster cells transformed by different primate papovaviruses. In this study, we showed that the peptide fingerprints of the protein isolated from cells transformed by SV40, BK and JC viruses were indistinguishable.

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174. MOLECULAR CLONING AND EXPRESSION OF SV40 SMALL-T AND LARGE-T ANTIGENS FOR EXPRESSION BACTERIA - MODEL FOR PRODUCTION OF HUMAN INTERFERON

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OBJECTIVES: 1. To clone the SV40 small-t antigen gene isolated from SV40 DNA into a plasmid vector (pBR322) for transformation of E. coli x1776 and expression of SV40 small-t antigen. 2. To clone the SV40 large-T antigen gene obtained by reverse transcription of the processed poly A containing mRNA for large-T into a plasmid vector (pBR322) for transformation of E. coli x1776 and expression of SV40 large-T antigen. 3. Using the above as model, to clone a human interferon gene into the plasmid vector (pBR322) for transformation of E. coli x1776 and expression of human interferon.

APPROACH: Many attempts to clone a eukaryotic gene into a cloning vector for specific expression of the eukaryotic protein have not met with success thus far. We believe that the most likely explanation for this is that the eukaryotic mRNAs lack the appropriate initiation signals for recognition by prokaryotic ribosomes. We propose to chemically synthesize an E. coli ribosome binding (initiation) site, and attach it in front of the eukaryotic SV40, t, T and human interferon genes prior to their insertion into the cloning vector. This we hope will assure their expression in bacteria.

175. ISOLATION AND FUNCTION OF SMALL SV40 T ANTIGEN

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During the past grant period, we have succeeded in isolating bacterial clones which synthesize small-t antigen in an apparently intact state. The only difference between the molecule isolated from the prokaryotic host and eukaryotic cells is the absence of an N-acetyl group in the former case. The molecule is now under study with respect to its chromatographic and sedimentation behavior as part of an effort to purify it to homogeneity.

176. CLONING AND TRANSLATION OF SV40 LARGE-T (SMALL-T) ANTIGEN GENES

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No summary has been provided to the Smithsonian Science Information Exchange.

177. USE OF XENOPUS OOCYTES AND EGGS FOR IDENTIFICATION OF FUNCTIONAL COMPONENTS OF T ANTIGEN IN SV40 GENE EXPRESSION

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The objectives of the proposed research are to identify separate biological functions of T antigens by injection of Xenopus oocytes and eggs with DNA from SV40 early gene deletion mutants. In addition, we hope to localize an early gene deletion on the SV40 genome responsible for certain biological properties associated with T antigens. The specific aims of this proposal are: 1) To identify separate functional properties of T antigen by injecting purified DNA from various SV40 early gene deletion mutants into frog eggs or oocytes from the species of Xenopus laevis. a) To compare the ability of various early gene deletion mutants of SV40 to initiate viral specific replication upon injection. b) To compare the ability of early gene deletion mutants to transcribe SV40 late mRNA upon injection. c) To compare amounts of early mRNA synthesis between early gene deletion mutants and wild type SV40 DNA upon injection. 2) To examine biological activities of T antigens, when T antigens and DNA from SV40 early gene deletion mutants are injected into Xenopus oocytes and eggs. 3) To map on the SV40 genome, the deletion responsible for a certain early gene function. 4) To examine which restriction endonuclease DNA fragments of SV40 DNA contain the promoter sequence for early gene transcription.

178. ISOLATION AND CHARACTERIZATION OF THE GENE CODING FOR SV40 MIDDLE-T ANTIGEN

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We propose to study in detail the nature of the gene(s) encoding the middle-t antigen of the human cells transformed by simian virus 40 (SV40). The aim of the initial stages of the project will be to clone, in prokaryotic vectors, reverse transcripts of mRNA(s) which code for the antigen, and to use these clones to isolate from a library of cloned fragments of human DNA, the genomic sequences which specify the antigen. Later phases of the work include sequence analyses of the genomic and cDNA clones, and comparison of the structures of the genes and their mRNAs. In this way we shall determine whether intervening sequences are present in the natural middle-t antigen gene. This availability of well characterized clones carrying middle-t sequences will open up many future possibilities, which include: a) Obtaining expression of the gene in prokaryotes, b) studies of the mechanism by which the concentration of cellular middle-t antigen is greatly elevated in response to viral large T antigen, c) re-introducing the gene to untransformed mammalian cells.

179. THE SV40 TUMOR ANTIGEN

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We are using a battery of biochemical techniques as well as recombinant DNA methods to determine how the large T-antigen of SV40 regulates gene expression and effects neoplastic transformation.

Our studies indicate that large T-antigen interacts with the major groove of the SV40 DNA at three tandem binding sites in a sequential manner. More recently, we have shown that large T-antigen is an active enzyme capable of hydrolyzing ATP at a rate of 0.6 micromoles/hr/mg. The T-antigen ATPase is rapidly inhibited by monoclonal antibodies directed against large T and is greatly stimulated by poly d T. Moreover, homogeneously pure large T-antigen is able to bind specifically to a 54,000 dalton cellular phosphoprotein found in transformed cells but not detectable in normal cells. Finally, we have shown that small amounts of purified T-antigen can stimulate the production of sensitized lymphocytes capable of specifically killing SV40 transformed cells in vitro.

180. REGULATION OF IN VITRO TRANSCRIPTION OF SV40 BY T-ANTIGEN

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The SV40 gene A product, large T-antigen, is a protein of 96,000 daltons which is expressed early after infection of host cells. Large T-antigen is required for the establishment of the transformed phenotype in nonpermissive hosts and plays a regulatory role in initiating viral DNA replication and transcription in permissive hosts. Experiments in vivo with temperature-sensitive gene A mutants show that early viral RNA is overproduced at nonpermissive temperatures, indicating that T-antigen may regulate its own transcription. Recent work by my sponsor has shown that T-antigen binds selectively to three sites on SV40 which cover the region where transcription of the SV40 early genes is thought to originate. This suggests that T-antigen may regulate RNA synthesis by interacting directly with a region of DNA adjacent to or overlapping the RNA polymerase initiation site(s).

I propose to study the transcriptional regulation of SV40 genes in vitro by using cloned DNA templates and a combination of purified RNA polymerase II and partially fractionated animal cell extracts. First, I plan to demonstrate that faithful transcription of SV40 genes can be obtained reproducibly by this in vitro reconstituted system. This will be accomplished by using DNA templates of short and defined length so that a 'run-off' transcript originating from a putative 'promoter' site can easily be distinguished from background transcriptional products on the basis of electrophoretic mobility. Next, I intend to characterize and map the primary transcripts synthesized in vitro to confirm the identity of these discrete RNA molecules. A combination of methods including hybridization of RNA products with restriction fragments of the SV40 early genes and T1-oligonucleotide determination will be used. My major effort will then be directed towards manipulating the in vitro system so that transcription of the SV40 genes can be regulated by exogenously added purified large T-antigen. Finally, it may be possible to use cloned DNA templates containing mutant T-antigen binding sites and/or RNA polymerase initiation sites to characterize further the regulatory mechanism of transcriptional control in SV40.

181. BIOLOGY OF SV40 SPECIFIC TRANSPLANTATION ANTIGEN

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The overall objective of the proposed research is to determine the role of specific viral genes in the expression and maintenance of the transplantation rejection antigen (TrAg) in cells infected with or transformed by papovavirus SV40. We have recently shown that a functional A gene product, which is known to be involved in the initiation and maintenance of cell transformation, is required for the expression of TrAg. We now propose to further explore the role of the viral genome in the expression of TrAg by investigating: (1) The expression of TrAg in permissive cells infected with mutants of SV40 and relationship to the map location of the mutants. (2) Expression of TrAg in tsA transformed cells and its relationship to various parameters of the transformed cell phenotype. (3) The relationship of TrAg to the A protein and to T antigen. (4) The nature of TrAg cell surface receptors. (5) Cross-reactivity between SV40 TrAg and TrAg in cells transformed by human papovaviruses. A determination of the role of SV40 genes in the expression and maintenance of TrAg and of the interaction of this antigen with the cell surface is of considerable importance in expanding understanding of viral oncogenesis and immunosurveillance mechanisms which mediate the elimination of potential tumor cells.

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182. THE ROLE OF SV40 GENE A IN MAINTENANCE OF TRANSFORMATION

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The SV40 gene A codes for three proteins, large T, small T and tumor specific transplantation antigen (TSTA). The function(s) of these proteins in maintenance of the standard transformed phenotype are unclear. mKSA207, a tsA transformant of mouse kidney cells, is uniquely suited to study this problem because: (a) it is temperature dependent for the expression of transformed growth properties; (b) it can be depleted of intranuclear T antigen, and (c) at the nonpermissive temperature (39.8 degrees) newly synthesized T antigen accumulates in the cytoplasm. Cytoplasmic T antigen will be extracted from mKSA207 cells at 39.8 degrees, partially purified, and characterized as to: (a) size in SDS-polyacrylamide gels; (b) state of aggregation; and (c) heat stability of TSTA, T antigenicity, and DNA binding capacity. Nuclear extracts of mKSA207 at 39.8 degrees are being studied to see whether any gene A products are transported to the nucleus. TSTA activity is being assayed by tumor rejection test using a transplantable SV40 tumor line. Cytosol, nuclear, and membrane fractions from mKSA207 at 39.8 degrees are being studied to learn whether TSTA activity is associated with cytoplasmic T antigen, is transported to the nucleus, or is translocated to the membrane. Results obtained with mKSA207 are being compared with those obtained with heat-adapted mKSA207 cells which are temperature independent for expression of transformed phenotype but still display cytoplasmic T antigen at 39.8 degrees.

183. PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST SV40 TUMOR ANTIGENS

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To understand the functions of the variety of tumor antigens found in SV40-transformed cells it would be useful to have antibodies that recognize both unique and common determinants on these proteins. In an attempt to prepare such antibodies, the method of Kohler and Milstein has been employed. This technique allows the production of monoclonal antibodies directed against a single antigenic determinant. Spleen cells from mice bearing SV40-induced tumors were fused to a HAT-sensitive myeloma line NS-1. Immunoglobulin-secreting hybrids from several separate fusions have been analyzed for the production of antibodies against determinants unique to large-T as well as one hybrid producing antibodies to a determinant common to large-T and small-t have been found. These hybridomas are being cloned and the immunoglobulins they produce are being characterized.

184. MECHANISM OF THE MITOGENIC ACTIVITY OF SV40 AND POLYOMA VIRUS TUMOR ANTIGENS

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OBJECTIVE: The goal of our investigations is to define the cellular processes which are activated by tumor antigens of small DNA replication and mitosis in lytically or abortively infected cells. Specifically, we are interested in those reactions which are switched on early after infection at times when the production of the virus-coded tumor antigens starts (which is probably earlier than the time when tumor antigens can be detected in infected cells by immunofluorescence). In a second project we are trying, using in vitro DNA replication systems, to trace those proteins which are necessary for the initiation of DNA replication itself, in hope of being able to define the requirements for the synthesis of these proteins.

APPROACH: We use primary mouse kidney cells which are in a resting state prior to infection. Infection of these cells with polyoma virus leads to a lytic response with an eventual destruction of cells. Prior to this, cellular DNA synthesis is initiated at about the same time as viral DNA is being replicated. Cells start an abortive mitosis which is not completed because of the lytic effect of the virus. Infection with SV40 also causes induction of cellular DNA synthesis, there is no virus DNA replication and most cells go through one division cycle. In both systems effects of virus infections are studied up to the time of the initiation of DNA replication. Chromatin structure, transcription and translation are investigated at different times after infection in order to eventually define the cellular changes induced by tumor antigens.

185. CENTRIOLAR ANTIGENS AND INVOLVEMENT IN CELL PROLIFERATION

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We have found that infection of TC7 cells, African green monkey kidney cells, with simian virus 40 stimulates host antigens which locate in the centriolar region of cells. The centriolar antigens consist of two polypeptides with molecular weight of about 14K and 17K daltons. The stimulation of the antigens is a result of de novo protein synthesis which occurs very early in infection, 0 to 5 hours after infection. The centriolar antigens are induced in cells infected with temperature-sensitive mutants of all complementation groups (tsA, tsB, tsC, tsBC and tsD) at restrictive temperature but not in cells infected with viable deletion mutants which contain deletions in the region of small-t gene (0.54-0.59 map units) and exhibit reduced transforming ability. Antigenically related protein(s) exists in ciliated epithelial cells isolated from chicken trachea and the related antigen(s) is localized at basal bodies of the cells. Our specific aims are: (1) To study the nature of the antigens; (2) to study the nature of the antigen stimulation; (3) to study the involvement of the antigens in various transformed cells and in cells at the particular phase of cell cycle; (4) to study the presence of the related antigens in other cells; and (5) to study the localization of antigens at the supramolecular level.

186. IMMUNOCYTOCHEMISTRY OF HUMAN BRAIN TUMORS

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We have screened over 150 human brain tumors for the presence or absence of SV40 T-antigen. Two tumors were highly suspicious for the presence of T-antigen: one a human ependymoblastoma, the other a choroid plexus papilloma. In addition, we have developed a radioimmunoassay for T protein, which is applicable to extracts of human tumors. In addition, our immunocytochemical studies have been extended to include the identification of various hormones and pituitary tumors. This includes growth hormone, ACTH, and prolactin. We have developed a frozen section method, which will be applicable to the study of human pituitary tumors removed at surgery. We have concluded that T-antigen is not a significant component of human tumors, particularly meningiomas, but that a frozen section technique may have application in surgical treatment of pituitary adenomas.

187. SV40 CELLULAR ANTIGENS AND HOST RANGE

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This proposal is divided into two sets of experimental approaches designed to provide a better understanding of gene regulation in developmental systems. In these studies SV40, polyoma and the adenoviruses are used as probes to elucidate viral host range limitations in teratocarcinoma cells and alter cellular gene expression by studying the production of cellular tumor antigens after virus infection.

I) Host range restrictions - polyoma and SV40 express their early proteins in most mouse cells in culture. Both viruses fail to produce these proteins in murine embryonal carcinoma cells (EC cells) which are the stem cells of teratocarcinomas. Studies to date indicate that EC cells infected with these viruses either fail to transcribe the early viral genes or fail to process the viral nuclear RNA into spliced mRNA species. Experiments are designed to study the following aspects of the mechanism of this host range restriction: 1) At the level of transcription and RNA processing, 2) a study of the RNA polymerase II from EC cells, 3) the isolation of virus host range mutants that grow

on EC cells, 4) temperature-sensitive EC cells' host range restriction, 5) a survey of EC cell restrictions and virus.

II) EC cells and papovavirus-infected and -transformed cells express high levels of a cellular protein that is specifically immunoprecipitated by sera from animals bearing SV40-induced tumors. This cellular tumor antigen is physically complexed with SV40 large T antigen in solution. Experiments are designed to 1) study the nature of this complex with purified proteins, 2) examine the effect of the cellular antigen on T-antigen DNA binding and enzymatic activity, 3) isolate mutants in the gene coding for this cellular tumor antigen, 4) study the expression of this cellular protein in developmental systems, and 5) characterize additional tumor antigens recently detected in EC cells and transformed cells.

F. ADENOVIRUS AND POLYOMA VIRUS ANTIGENS

188. CHARACTERIZATION OF SURFACE ANTIGENS OF HUMAN ADENOVIRUS TYPE 12-TRANSFORMED AND TUMOR-DERIVED HAMSTER CELL LINES, HT-4 AND HG-1

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OBJECTIVE: Isolation of a virus-induced cell surface antigen(s) (S antigen(s)) from hamster cells transformed by human adenovirus type 12 (Ad 12) and its biochemical, biological and immunological characterization, with particular reference to its relation to TSTA activity.

APPROACH: S antigen(s) is extracted with Nonidet P 40, isolated from Ad12-transformed cell lines (HT-4 and HG-1) by affinity chromatography using antiserum against S antigen(s), analyzed by SDS-PAGE and by rosette formation inhibition technique, and assayed for its antigenicity, and its immunogenicity will be assayed by tumor transplant rejection method.

PROGRESS: S antigen(s) has been purified from Ad12 tumor-derived cells (HG-1 line), stained with Coomassie brilliant blue as a 15 K band in SDS-PAGE, and shown to inhibit rosette formation in antiserum, S antigen-positive cells and protein A-coated sheep red blood cells.

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189. ADENOVIRUS T, SURFACE ANTIGENS AND TUMORIGENICITY

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Human adenoviruses have well-documented oncogenic potential in experimental animals and in vitro. Adenoviruses are not viewed as candidates for a major etiological role in human malignancy but they serve as a convenient probe in tumor biology. The transforming capacity of adenoviruses is associated with the left hand end of their genome which codes for transformation proteins. The products of this region of adenovirus type 12 genome will be isolated and characterized. We will attempt to define the mechanisms by which these products establish oncogenic transformation. Although all adenoviruses can transform cells in vitro, in vivo oncogenic potential of different serotypes varies from highly oncogenic to nononcogenic. The adenovirus-specific surface antigens will be identified in transformed cells and their role in the induction of the immune response will be analyzed. The immune response to cells transformed with highly oncogenic and nononcogenic adenoviruses will be qualitatively and quantitatively compared in syngeneic hosts. Data will be obtained indicating whether the immune response plays a major role in differences in tumorigenicity of individual adenoviruses in vivo.

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190. STUDIES OF VIRAL ANTIGENS IN VIRUS-INDUCED TUMORS

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The initial objectives of this project were to use human adenoviruses and adenovirus-SV40(Ad2-240) recombinants as tools to study the genetics of DNA tumor viruses, to define the role of viral genes and viral antigens in viral oncogenesis and to study the biology of Ad2-SV40 recombinants. Due to the lack of proper containment facilities in NIAID between January 1973 and May 1978, there has been a 5-year disruption (see past reports) in the major thrust of this project. During this interval, a study of Ad2-SV40 recombinant-associated tumor induction by these agents with the incorporation into the adenovirus 2 chromosome of a specific segment of SV40 DNA. To begin to understand the mechanism by which this SV40 DNA segment conveys oncogenicity to these recombinants, it was necessary to understand why Ad2 was nononcogenic for hamsters. Since Ad2 will transform hamster cells in tissue culture, we initiated a study of the oncogenic properties of Ad2 transformed cells. The results thus far lead us to suspect that unrecognized host immune mechanisms in the hamster reject Ad2 transformed cells and transformed cell-induced tumors. Such a concept suggests that the presence of a specific segment of the SV40 genome might induce functions in hamster tumor cells that interfere with the rejection process. Future studies will be directed toward more carefully defining these concepts.

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191. AUGMENTATION OF IMMUNOGENICITY OF TUMOR CELLS BY DNA VIRUS TRANSFORMATION

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Successful tumor immunity relies upon an appropriate host recognition and response to tumor-specific antigens. Progressive tumor growth in animals is associated with an immune response which is weak, inappropriate, or actually protective of the tumor cells. The goal of this research project is to augment the immunogenicity of murine and human tumor cells by secondarily transforming the cells in vitro with oncogenic DNA viruses (SV40, adenovirus 12, and polyma). Thus, by introducing new antigens onto the cell membrane of the tumor cell it is anticipated that host animals will be directed to respond to the new membrane antigens (TSTA) and in addition to show a heightened response to the membrane-located tumor-specific antigens of the original tumor cell. Other reported models of augmenting the immunogenicity of tumor cells utilize treatment of the cells with concanavalin A, dinitrophenylaminocaproic acid, iodoacetate, or Vibrio cholerae neuraminidase. In addition, other studies have infected tumor cells with lytic viruses (myxoviruses, paramyxoviruses, or reoviruses) and have used the membranes from the lysed cells for immunization.

Once tumor control or regression has been achieved in vivo, it will be the goal of this project to study the parameters of the immune response in the regressor animals as compared with the immune responses in progressor animals.

192. STRUCTURAL STUDIES ON POLYOMA VIRUS TUMOR ANTIGENS

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Three tumor antigens (T-antigens) are expressed early during productive infection by Polyoma Virus (Py). Recently, a model for early viral genome expression based on DNA sequence, RNA mapping and peptide fingerprinting data obtained by other groups working at I.C.R.F. has been proposed. We have tested the prediction that all three T-antigens have the same amino terminal (N-terminal) amino acid sequences.

Earlier work in this laboratory using microsequence analysis of the two SV40 T-antigens has shown that these two proteins share N-terminal sequences. This technique, however, is expensive and time consuming and we have utilized a more simple and rapid technique, using fingerprint analysis to identify the N-terminal tryptic peptide of the Py T-antigens.

One of the two shared methionine-containing tryptic peptides present in fingerprints of Py large (L-T), middle (M-T) and small (S-t) T-antigens is resistant to aminopeptidase digestion, whereas all other peptides are degraded. We assume that this peptide is uniquely protected from enzymatic digestion by the presence of a modifying group on the variation-NH2 group. If this is the case then the resistant peptide must be the N-terminal tryptic peptide of the Py T-antigens.

The DNA sequence encoding the common N-terminal region predicts that the N-terminal tryptic peptide of the Py T-antigens is Met.Asp.Arg. Digestion of the Py T-antigens with an arginine-specific enzyme from submaxillary gland releases the putative N-terminal peptide. These data, together with the finding that the peptide has a mobility similar to, but not identical with, the N-terminal tryptic peptide of SV40 T-antigens, strongly suggest that Py T-antigens begin N-acetyl.Met.Asp.Arg.

The finding that the three Py T-antigens share N-terminal amino acid sequences and consequently that M-T does not have a signal sequence raises questions about the mechanism whereby M-T becomes associated with the plasma membrane.

193. ANALYSIS OF RELATIONSHIP BETWEEN POLYOMA-INDUCED SURFACE ANTIGEN AND ONCOFETAL ANTIGENS AND H-2 ANTIGENS

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The summary for this project is under entry no. 61.

194. ISOLATION AND ANALYSIS OF POLYOMA VIRUS TUMOR ANTIGENS

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Polyoma virus is capable of inducing cell transformation in vitro and is tumorigenic in the appropriate animal host. That portion of the viral genome which is important for these events has been identified and has been intensively studied for the past several years. Three polyoma specific proteins coded for by this region were first detected immunologically and defined as tumor antigens. At early times after polyma virus infection of susceptible mouse cells, viral RNA molecules are transcribed with a single initiation codon used for the expression of all three proteins. RNA processing events result in the removal of termination codons and the generation of diverse mRNA molecules.

The objective of this proposal is to clone coding sequences from purified RNAs by reverse transcription, for the polyoma early proteins, in an appropriate plasmid vector so that E. coli transcriptional and translational control elements may efficiently produce these proteins in the bacterial cell. Potential in vitro and in vivo activities of the isolated tumor antigens (for example, protein kinase or ATPase properties, or binding to DNA or cellular macromolecules) will be assayed in hopes of elucidating their biological role. By this means, it is desired to clarify the molecular basis of this virally induced cell transformation.

195. CHARACTERIZATION OF THE MRNAS CODING FOR POLYOMA VIRUS TUMOR ANTIGENS PRESENT IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS

Mellor, A., Imperial Cancer Research Fund, Translation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

RNA extracted from productively infected 3T6 cells was enriched for Py sequences by hybridization to Py DNA covalently attached to cellulose. Polyadenylated RNA bound to Py DNA was fractionated on denaturing sucrose gradients and fractions were used to prime cell-free protein synthesis in a cell-free system derived from L-cells. mRNA activities encoding the three major early proteins, large (L-T), middle (M-T) and small (S-t). T-Antigens were located following immunoprecipitation of the cell-free products using Pyanti-tumor serum. The results show that mRNA encoding L-T is smaller (about 20.5S) than mRNAs encoding M-T and S-t (about 21:5S) which are not separated by this procedure. These findings are consistent with the current understanding of early mRNA structure, as determined by S1 mapping (see Report 65) and of its functions deduced from the recently proposed model for Py early gene expression (see Report 87). In this model, all three early proteins are encoded by spliced mRNA molecules. M-T and S-t mRNA are of similar sizes (due to the similar sizes of proposed spliced regions) and L-T mRNA is smaller (due to a more extensive spliced region).

This technique has also been used to size the early viral mRNAs expressed in a rat cell line transformed by Py (82-rat, see Report 82). The single insert of viral DNA in the chromosome of these cells has been mapped by restriction enzyme analysis and the structures of the early viral RNA molecules have been determined by S1 mapping (see Reports 78 and 65, respectively). The results of this size analysis were used to correlate protein coding functions with RNA structure determined by S1 mapping analysis and thus to deduce which regions of DNA in the integrated viral genome are expressed as protein. For example, a T-antigen-related protein of 7,000 daltons is encoded by an RNA molecule sedimenting at 18-19S and is transcribed from the left hand early region of the integrated viral genome in 82-rat cells.

It is hoped that this approach will be used to study early gene expression in other Py transformed cell lines containing integrated viral genomes that have been mapped.

196. PROTEIN KINASE ACTIVITY ASSOCIATED WITH POLYOMA VIRUS MIDDLE-T ANTIGEN

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In an attempt to ascribe biological functions to the tumor antigens of polyoma virus (Py), we have asked whether these proteins have an associated protein kinase activity, similar to that associated with the src gene product of the RNA tumor viruses. Using the assay described by Collett and Erikson (Proceedings of the National Academy of Science, USA, 1978, 75, 2021) and using antiserum raised against Py-transformed cells (anti-T serum) we can detect a protein kinase activity in immunoprecipitates of extracts of Py-infected cells. The activity is not present in uninfected cells, nor is it detected using control serum. Using anti-T serum raised in rats, the proteins phosphorylated in vitro by the kinase are Py middle-T and rat IgG heavy chain; using hamster anti-T serum only middle-T is phosphorylated.

Experiments using a number of mutants in the early region of Py DNA show that the kinase activity is associated with Py middle-T. Thus the amount of kinase activity detected is under the control of the viral A gene but the activity itself is not temperature-sensitive when isolated from tsA-infected cells. The activity is affected by mutations in the hr-t region and in the region shared by large-T and middle-T. The kinase activity, like middle-t, is present in purified preparations of plasma membranes.

The kinase is also detected in Py-transformed cells but not in untransformed cells nor cells transformed by other viruses. Some of the Py-transformed cells that contain kinase activity lack full-sized Py large-T but all contain middle-T. The activity is not detected in a cell line (18.37) which contains integrated viral DNA of a non-transforming hr-t mutant, and which contains Py large-T but not middle-T nor small-t. Together all these results show that Py middle-T or a protein which specifically binds to it has protein kinase activity in vitro, and they raise the possiblility that protein kinases play an essential role in papovavirus-induced transformation.

So far, attempts to demonstrate phosphorylation of middle-T in vivo have been ambiguous. Structural studies show that the phosphate residue attached to Py middle-T in vitro is present in the carboxy terminal half of the molecule, but we have not yet shown unequivocally that similar modification occurs in vivo. (Text Abridged.)

197. MACROMOLECULAR SYNTHESIS AND CELL GROWTH CONTROL

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OBJECTIVES: Firstly, to understand the way in which polyoma virus causes cellular transformation. To do this we will investigate the functions and subcellular locations of the three T antigens of polyoma virus - the 90,000, 60,000 and 22,000 dalton products of the early region of polyoma virus. By the use of monospecific sera and partial purification we hope to be able to define the roles of the individual T antigens.

Secondly, to investigate the mechanism by which the 60,000 dalton product of the Rous sarcoma virus sar gene (p60src) effects cellular transformation. p60src is a protein kinase with the unusual specificity of phosphorylating tyrosine. Our efforts will concentrate on identifying the intracellular targets of this protein kinase. This task will be aided by using the criterion that any substrate of p60src should contain the rare modified amino acid, phosphotyrosine.

BIBLIOGRAPHIC REFERENCES: Hunter, T., M.A. Hutchinson, W. Eckhart, T. Friendmann, A. Esty, P. LaPorte and P. Deininger. Regions of the polyoma genome coding for T antigens. Nucl. Acid Res., 7:2275-2288 (1979). Sefton, B.M., T. Hunter and K. Beemon. Temperature-sensitive transformation by Rous sarcoma virus and temperature-sensitivie protein kinase activity. J. Virol., 33:220-229 (1980).

198. VIRAL TRANSFORMING PROTEINS

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The aim of this project is to further our understanding of the way in which polyoma virus causes malignant transformation. Expression of the 'early' region of polyoma virus is required to initiate and maintain the transformed state. The early region of polyoma virus codes for three related proteins (T antigens) - large T antigen, medium T antigen and small T antigen. The large T antigen is required to initiate transformation but not usually for maintenance. Both the medium and the small T antigens appear to play roles in maintaining the transformed state. We plan to purify each of the three T antigens, both from polyoma virus transformed cells, and, if possible, from bacteria expressing recombinant DNA clones containing copies of the mRNAs for the three T antigens. We propose to investigate the structures and test the functions of the purified T antigens. In particular we will examine the role of the large T antigen in the initiation of viral DNA synthesis. In immunoprecipitates containing polyoma virus T antigens, there is preferential phosphorylation of the medium T antigen upon addition of labeled ATP. We will ask whether the purified medium T antigen itself possesses a protein kinase activity or whether there is a cellular protein kinase specifically associated with the medium T antigen. This answer to this question is especially important because it is already known that the transforming protein of Rous sarcoma virus is a protein kinase. The predicted amino acid sequence of the small T antigens has homology with certain glycoprotein hormones. We will measure whether the purified small T antigens has growth factor activity. In this manner we hope to define the ways in which the transforming proteins of polyoma virus interact with the cell to cause malignant transformation.

199. IMMUNE RESPONSE TO SURFACE ANTIGENS IN BK VIRUS INDUCED TUMOR CELLS

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BK virus induced hamster tumor cells will be used to demonstrate immune response and the presence of a tumor specific surface antigen. Previously transplantation rejection tests were used to show the presence of a tumor specific transplantation antigen (TSTA) in BKV-induced hamster tumor cells. Preliminary results suggest that a tumor specific surface antigen in BKV tumor cells can be demonstrated by cytotoxicity tests. The 51 Cr release assay (Brunner et al., 1976) will be used for the quantitative measurement of cell mediated cytolysis in vitro.

200. MECHANISM OF THE MITOGENIC ACTIVITY OF SV40 AND POLYOMA VIRUS TUMOR ANTIGENS

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The summary for this project is under entry no. 184.

201. VIRAL GENE FUNCTIONS AND REGULATION OF CELL GROWTH

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Our objective is to clarify the role of polyoma T antigens in cell transformation. We will isolate mutants affecting the medium T antigen of polyoma. We will construct recombinant DNAs to allow the T antigens to be synthesized in bacteria, or in high amounts in mammalian cells. We will study functions associated with the T antigens, particularly a protein kinase activity of unusual specificity in polyoma T antigen immunoprecipitates. We will seek to relate T antigen functions to mechanisms of cell growth control by studying the control of transcription in polyoma infected and transformed cells, and early viral functions such as mitogenic activity and events leading to anchorage-independent growth.

BIBLIOGRAPHIC REFERENCES: Hutchinson, M.A., Hunter, T. and Eckhart, W. Characterization of T-antigens in polyoma-infected and transformed cells. Cell, 15, 65-77 (1978). Hunter, T., Hutchinson, M.A. and Eckhart, W. Translation of polyoma virus T antigens in vitro. Proc. Nat. Acad. Sci. USA, 75, 5917-5921 (1978).

202. ANTIGENS INDUCED BY POLYOMA VIRUS TRANSFORMATION

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Polyoma virus transformation of mammalian cells is associated with the appearance of new cell associated protein antigens which are of major importance in the immune rejection of such cells. In addition, these molecules probably provide the molecular basis for the induction and expression of some of the tumor cell-like properties associated with transformed cells.

It is proposed to study the molecular nature of polyoma virus induced antigens in more detail with special attention being given to the antigenic changes occurring at the cell surface. This is to be accomplished by immunoprecipitation, gel electrophoresis and autoradiography of radiolabeled antigens prepared from extracts of radiolabeled cells.

Using similar techniques, we plan to explore the molecular basis for the observed in vitro cross-reactivity between SV40 transformed and polyoma transformed cells which is presumably due to some immunologically similar antigen present on the surfaces of both types of cells. Studies of transformed cells of different species will likewise be carried out to obtain additional information on the surface antigens of transformed cells to determine which of these are specified by the species of transforming virus and which are influenced by species of cell origin.

The in vivo activities of antigen preparations will also be explored.

G. OTHER VIRUS-RELATED ANTIGENS

203. HEPATITIS B VIRUS AND HEPATOCELLULAR CANCER

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The distribution of hepatitis B-associated antigen and antibody and the incidence of hepatocellular cancer in Yamaguchi Prefecture are being examined. The data obtained will be compared with those from Taiwan where there are a greater number of hepatitis B antigen carriers and a higher incidence of hepatocellular cancer in the population.

204. HUMAN HEPATOMA CELL LINE PRODUCING HEPATITIS B SURFACE ANTIGEN

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This is part of a broader project. A summary of this subproject is not available.

205. DETECTION OF RNA TUMOR VIRAL ANTIGENS IN HUMAN PROSTATE

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Human malignant and benign prostatic tissues are obtained from surgery and established in monolayer culture as well as in an animal model, the athymic nude mouse. Extracts are prepared from the surgical tissues and from the in vitro grown cells and are examined by competition radioimmunoassay for the presence of antigens which react with antisera prepared against antigenic determinants common to RNA tumor viruses. Extracts demonstrating viral antigenic activity are further analyzed by a three-phased, two-dimensional immunoelectrophoresis system designed to size and isolate the reacting proteins. Patient sera are also collected and analyzed for immunologic responses to prostatic tissue extracts in radioimmunoassays and for reactions with isolated interspecies viral antigens. Localization of viral-like proteins on the surfaces of prostate cells is determined by peroxidase and ferritin labeling utilizing the electron microscope. Pronase extracts are prepared from the tumor removed from athymic nude mice and prostate-specific proteins are identified by column chromatography, slab gel electrophoresis and isoelectric focusing. Heterologous rabbit antisera are then raised to these isolated prostate-specific proteins. The heterologous serum reactions are also compared to the patient serum reactions against the same proteins.

206. SEARCH FOR ONCOGENIC VIRUSES IN HUMAN PROSTATE CANCER

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Immunological studies on prostatic carcinoma will focus on a viral etiology. Three main areas will be developed: (1) Detection of the presence and characterization of oncornavirus-like antigens in prostatic carcinoma, benign prostatic hyperplasia and normal prostate; (2) examination of human prostate tissue for the presence of specific antigenic markers and the relationship of these antigens to viral antigens; (3) determination of the presence of antibodies against oncornavirus antigens in sera of patients with prostatic carcinoma and benign prostatic hyperplasia, and determination of the major auto- or allo-antigens associated with prostatic carcinoma. The methodology will utilize appropriate techniques to arrive at general and specific answers. These techniques will include the radioimmunoassay and immunofluorescence for detection of type C virus-like antigens. Extraction procedures, such as the 3M potassium chloride method and other methods, will be used for the preparation of membrane antigens. Antigens thus obtained may be detected by Ouchterlony method and again by the radioimmunoassay and immunofluorescence methods. For characterization of antibodies in sera of patients, some of the above-mentioned methods will be used, as well as immunoperoxidase procedures. Similar studies will also be carried out with sera of rats bearing transplanted prostate tumors and with tissues of these rat prostate tumors.

207. IMMUNOGENICTTY STUDIES WITH ANTIGEN-COATED LIPOSOMES

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DESCRIPTION: Artificial phospholipid vesicles (liposomes) will be coated with various cellular membrane antigens, in particular with purified viral antigens and with histocompatibility antigens. We want to determine the structural prerequisites for the induction of cellular or humoral immune reactions against membrane-bound antigens (antigen density, the mode of integration of the antigen into the membrane, hapten and carrier determinants). Liposomes will also be used to study the important questions of possible interactions of viral antigens and histocompatibility antigens in cellular membranes. Finally such liposomes, coated with viral antigens (virosomes), shall be used as a new type of vaccine. Such vaccines would be of great importance for human application since they do not contain the potentially dangerous nucleic acid of conventional vaccines.

208. ANTIBODY RESPONSES TO VIRAL ANTIGENS

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To understand further the regulation of the immune reponse to viral antigens we have induced, in vivo, antibody responses to myxoviruses. PFC responses were measured using target cells coated with virus. We found that virus-specific responses may be obscured by another large response resulting from a neuraminidase effect on the target cells. Mice responding to Newcastle Disease virus produced up to 150,000 plaque-forming cells (PFC) per spleen to neuraminidase-treated sheep red blood cells (SRBC). Murine tumor cells, thymocytes and red cells also served as targets when similarly treated but were ten-fold less sensitive. Nevertheless, specific reactions were observed using A/Jap Bel (2 times 10 to the fourth power PFC/spleen). Secondary responses could be induced in vitro using virus-coated stimulator cells. The significance of the autoantibody response and the regulation of the anti-viral response at the cellular level are continuing projects.

Human sera containing IgG able to bind primate retroviruses (HL23) have been further examined. Cell lines and erythrocytes from several species inhibit binding, as do some monosachrides. We conclude that binding is not specific for these viruses, but is directed rather at glycosyl groups present on virions but present also on many other proteins.

209. HUMAN WART VIRUS AS AN ONCOGENIC AGENT

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The purposes of the proposed work are: (1) To continue to study two human skin cell culture systems which thus far have shown interesting changes suggestive of transformation after inoculation with purified human wart virus. These studies would include characterization and identification of the non-virion cellular antigens already detected in these cultures by immunofluorescence and complement-fixation, search for evidence of the viral genome in these cells using biochemical techniques, and further trials of their tumorigenic capacity in an intact host. (2) To continue to investigate the non-virion new cellular antigens (neoantigens) which appear to be virus induced and which also have been detected by immunofluorescence in sections of human warts and condylomata acuminata employing sera from certain types of wart patients and experimental guinea pig sera. (3) To apply these sera in search of similar wart virus-induced antigens in a variety of human epidermal tumors. (4) To attempt to reproduce the experiments of Eisinger et al. who reported the in vitro production of a cytolytic infection with wart virus. In these experiments the appearance of mature virions as well as of non-virion new antigens will be followed. (5) To study the role of cell-mediated immunity in human wart virus infections, especially the reactions of T and B lymphocytes and macrophages in patients with limited as opposed to widespread evidence of disease and in those undergoing spontaneous regression.

210. CELLULAR IMMUNITY TO TUMOR ANTIGENS

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OBJECTIVES: Utilizing the cellular immunity test, establish the specificity of antigens which exist on human tumors, particularly of those antigens which are associated with possible oncogenic viruses. Study the epidemiology of cellular immunity to these virus-associated tumor antigens. Study the linkage of HL-A antigens to susceptibility to tumors. Perform HL-A testing for other VCP investigators as directed by the Project Officer.

IV. ANTIGENS ASSOCIATED WITH CANCERS BY TYPE OR SITE

A. ANTIGENS ASSOCIATED WITH LEUKEMIA AND LYMPHOCYTES

211. HUMAN LEUKEMIA AND LYMPHOMA - LYMPHOCYTE MEMBRANE ALLOANTIGENS AND MEMBRANE MARKERS (ABBREV)

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OBJECTIVE: Investigation of the serological behavior of lymphoid and/or blastic cells from leukemic patients with human alloantisera, especially those containing anti-HLA-DR antibodies. Correlation with other membrane markers, leukemia or lymphoma types and clinical outcome.

APPROACH: Peripheral blood lymphocytes and/or leucoblasts (and, when available, lymphoid cells from lymph nodes, bone marrow, etc.) from both acute and chronic leukemia and from various lymphomas are studied in terms of HLA-A,B,DR typing, by complement-dependent microlymphocytotoxicity test; T or B membrane markers, including surface immunoglobulins, human T lymphocyte antigen (HLTA), receptors for Fc fragments, complement, mouse red blood cells and sheep red blood cells; their ability to detect low levels of various antibodies in human alloantisera.

PROGRESS: Preliminary results suggest that there does not seem to be any striking difference between the HLA-DR antigen frequencies of the different types of leukemic patients and healthy blood donors. Studies comparing the expressivity of these antigens and the other membrane markers in the different types of leukemia are in progress.

212. IMMUNOCHEMICAL STUDIES OF LYMPHOCYTE MEMBRANE MARKERS IN LYMPHOBLASTIC LEUKEMIAS - CLINICAL IMPLICATIONS

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The project is aimed at the study of some lymphocyte membrane markers in lymphoid cells (peripheral and bone marrow) in the course of acute lymphoblastic leukemia.

Reasons for undertaking such work lie mainly in the availability of a test procedure for DR antigen quantitation, given the role of differentiation antigens currently assumed for such membrane specificities. A radioassay is employed, based on the binding of heterologous antisera to partially purified and radioiodinated DR antigens (obtained from B lymphoblastoid cells or cell lines). The work is done in collaboration with the Pediatrics Department of the same Medical School where protocol No. 7601 for therapy of acute lymphoblastic leukemias of the 'Societa Italiana contro le leucemie' is followed.

Conventional staining procedures and lymphocyte membrane markers are used to classify the disease and to define its different stages.

Another approach involves HLA (A, B, C and DR loci) typing of patients and of their parents in order to search for possible HLA and disease associations and to delineate the pattern of additional reactions often observed in such patients.

213. STABILITY OF LEUKEMIC PHENOTYPE DURING DISEASE PROGRESSION

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The stability of the leukemic maturation arrest position as reflected in the dominant cell phenotype has been investigated by comparing the expression of multiple antigenic and enzymatic markers on cells at presentation and in one or more subsequent relapses. This question is of relevance to attempts to monitor residual or re-emerging disease and to direct therapeutic manipulations at cells expressing particular cell surface antigens. Of 120 cases examined to date, 101 showed conservation of phenotype and 19 showed a phenotypic shift. Of the latter the most common alteration was in the loss or acquisition of the cALL membrane antigen. Whether these alterations are the result of intraclonal selection events, cell surface modulation or structural lesions associated with evolving aneuploidy is unknown. Several aneuploid ALL cell lines maintained in vitro for more than two years have shown no qualitative alterations in phenotype.

214. IDENTIFICATON OF NEOPLASTIC ANTIGENS AND IMMUNOLOGICAL FOLLOW-UP OF PATIENTS WITH LYMPHOMAS, LEUKEMIAS AND SOLID TUMORS

Del Giacco, G. S., Mantovani, G., Locci, F., Cengiarotti, L., Leone, A. L., Loy, M., Mura, E., Pau, L., Universita degli Studi di Cagliari, Chair of Clinical Immunology, *Cagliari, Italy*

Anti-lymphocyte antibodies have been found in the sera of patients with systemic lupus erythematosus (SLE). They belong to the IgM class, are active against T-cell bearing receptor for Fc of IgG (T.G.) and are not directed against HLA antigens. This suggests that an antigen present on lymphocyte membrane can be identified by some antibodies present in the sera of patients with active untreated SLE. Our objective has been to study by means of the active sera the cells obtained either from the blood of patients with acute lymphoblastic leukemia or from lymph nodes of patients with lymphomas. In one case of ALL with T blasts sera reacted with the majority of blasts suggesting that the antigen common to T.G. cells was present also on leukemic blasts. Another case of T ALL has been negative. Two cases of non-Hodgkin's lymphomas were negative with SLE sera when the cells of lymph nodes were studied. Studies to isolate the antigen are in progress.

The immunological follow-up has been performed studying the production of LIF by neoplastic patients when their lymphocytes were put in contact with neoplastic homogenates from different cancer tissues. Eighty percent of breast cancer patients had positive reactions but in 20% aspecific positive results were obtained.

The reactivity in vitro of T.G. from normal and neoplastic subjects when reacted with thymic hormones or levamisole has been studied. No increase in the percentage of T.G. has been documented.

The percentage of T.G. in the lymphs of the thoracic duct from patients with lung cancer has been studied. The percentage is lower than in peripheral blood of the same subjects and than in normal subjects, suggesting either a trapping of T.G. from tumor mass or a different recirculation.

215. TUMOR-ASSOCIATED ANTIGENS AND IMMUNOGENICITY OF HUMAN LEUKEMIC CELLS

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OBJECTIVES: (a) To identify and characterize cell surface antigens associated with acute myeloid leukemia in humans. (b) To understand the immune reactivity of patients to autologous leukemic blasts.

METHODS: Surface antigens will be defined using monoclonal antibodies produced by the Komler and Milstein hybridoma technique.

Immune reactivity will be studied using leukemic blasts and remission peripheral blood leukocytes, with and without the addition of soluble lymphokines, in vitro in the mixed leukocyte reaction (MLR) and the cell-mediated lysis (CML) assays.

PROGRESS: The project has just begun.

216. IA-LIKE ANTIGENS AND OTHER MEMBRANE GLYCOPROTEINS IN HUMAN LEUKEMIA

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OBJECTIVE: To use immunologic reagents to characterize and follow the progress of patients with chronic and acute leukemias. Reagents will include Ia-like anti-sera and sera against other developmentally related surface proteins.

APPROACH: Standard techniques of immunofluorescence and surface protein radiolabelling and analysis.

217. DETECTING PAUL-BUNNELL ANTIGEN IN LYMPHOMA AND LEUKEMIA

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We have recently reported the presence of Paul-Bunnell (PB) antigen in the spleens of patients with Hodgkin's, leukemia and other hematopoietic malignancies (K. Kano, A. Fjelde, F. Milgrom; J. Immun. 119:945, 1977), and at the present time we are engaged in studies concerning this antigen, which is found in the cells of other species including sheep, cows, horses and mice, but not normally in man. The (PB) antibody to this antigen appears in man during the acute stage of infectious mononucleosis (IM) and subsequently disappears. Although patients with Hodgkin's, leukemia and other hematopoietic diseases have the PB antigen, they do not apparently, like IM patients, produce PB antibodies. Using IM sera, we have detected and reported the presence of PB antigen, but have never identified in histological preparations or cell cultures the cell types associated with the presence of the antigen. We propose to employ autoradiography, using 125I - conjugated antibody from high titered IM convalescent sera in these studies. We are especially interested in Hodgkin's disease. The infiltrates of Hodgkin's disease are generally composed of a number of different kinds of cells, a characteristic cell being the Reed-Sternberg cell. Which of the cells are associated with the PB antigen (or the origin of the antigen) is at present not known. The specific aim of the proposed work is to identify the cell or cells.

218. ANTIGENS OF HUMAN LEUKEMIC CELLS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

219. SEARCH FOR LEUKEMIA ANTIGENS (HUMAN)

Thomas, E. D., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

This is part of a broader project. A summary of this subproject is not available.

220. LEUKEMIA ASSOCIATED ANTIGENS (HUMAN, RABBIT, PRIMATE, GOAT)

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This is part of a broader project. A summary of this subproject is not available.

221. FUTURE CHARACTERIZATION OF THE COMMON ALL

Sutherland, D. R., Newman, R. A., Imperial Cancer Research Fund, Membrane Immunology Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

Recent studies on the common Acute Lymphoblastic Leukemia (cALL)-associated antigen have shown that the plasma membrane and the previously studied secreted form of it (ICRF Scientific Report 1978 -report 226) are very similar, if not identical, to each other with respect to: (a) molecular weight of c. 100,000 daltons (both reduced and non-reduced); (b) isoelectric point (pI 5.6-5.8), using broad pH range IEF; (c) carbohydrate heterogeneity as evidenced by lectin binding characteristics.

Whilst only 50 percent of the cALL antigen binds to Lentil lectin (like Thy-1) virutally 100 percent binds to Ricin lectin (RCA1). The RCA binding form of the antigen could not be sub-fractionated by IEF over a narrow pH range (pH 4-6). Peptide mapping is currently being used to compare the lentil binding and lentil non-binding forms. Preliminary experiments using glycosidases indicate that the carbohydrate moiety accounts for about 7 percent of the molecular weight and is not involved in the antigenic site.

cALL antigen can now be affinity-purified from cell lysates in large quantities and this will enable us to show whether it is an integral membrane protein (cf. Ia) or merely membrane-associated (cf. fibronectin). To this end we intend to perform charge shift electrophoresis and nitrene labelling experiments.

We have previously observed anti-ALL positive normal cells in fetal, neonatal and regenerating bone marrow. Recently we have been able to isolate cALL antigen from these samples; this supports our earlier contention that, although this antigen is diagnostically useful as a leukemia-associated antigen, it is essentially a normal differentiation antigen of lymphocyte precursors.

222. HUMAN LEUKEMIA- AND LYMPHOMA-ASSOCIATED ANTIGENS

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The principal aim is to isolate, characterize and clinically utilize human leukemia-lymphoma-associated (HLL) antigens of human malignant cells. We are isolating large quantities of purified HLL antigens using cultured leukemia-lymphoma cells, so that we can carry out detailed studies on the immunological and chemical properties of HLL antigens. The antigens isolated will be useful in obtaining strong, specific antisera to HLL antigens. Such antisera will in turn be useful for diagnostic purposes.

The HLL antigens which we have been detecting are found on the surfaces of cells. We have also found much of HTL antigen activity on isolated cell membrane preparations. Therefore, we are isolating these antigens from cell membranes prepared from cultured leukemia-lymphoma cells. The cell membranes are treated with deoxycholate to solubilize the HL antigens. The solubilized antigens are fractionated by gel filtration and affinity chromatography.

223. MEMBRANE ANTIGENS FROM NORMAL AND LEUKEMIC LYMPHOCYTES

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Our major objective is to isolate and characterize abnormal membrane components that are specific to or associated with human leukemia cells or malignant lymphoma cells, as well as to isolate normal membrane components, especially normal alloantigenic components of human lymphocytes that are linked to immunologic functions. Particular effort is directed to identification and isolation of the human homologues of mouse TL (thymus-leukemia) antigens, mouse Ia (I-region-associated) antigens and mouse Ly antigens and to further characterization of HLA (A,B,C) antigens.

Human lymphoid cell lines, T-cell-type, B-cell-type and null-cell-type, are being used for this purpose, since they retain a variety of biological functions and represent a uniform cell population whose supply can be relatively unlimited in amount. We also plan to produce T-cell hybrid clones which possess known immunological functions and to use them for the present purpose. We also investigate similar cell surface antigens in cells of malignant cell lines derived from sarcoma and/or carcinoma which are available in this laboratory.

The very sensitive quantitative radioimmunoassay method involving the use of radiolabeled reference antigens which has been extensively employed by us in earlier studies facilitates identification and isolation of the membrane components.

BIBLIOGRAPHIC REFERENCES: Koyama, K., Fukunishi, T., Barcos, M., Tanigaki, N., and Pressman, D. Human Ia-like antigens in nonlymphoid organs. Immunology 38: 333, 1979. Tanigaki, N., Tokuyama, H., Fukunishi, T., Minowada, J., and Pressman, D. Human cell membrane components dominant in T cell lineage: Identification and characterization of human TL-like antigens. Accepted for publication in J. Immunol., 1979.

224. HUMAN LEUKEMIA ANTIGENS - ISOLATION AND CHARACTERIZATION

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The immunologic characterization of the leukemic cell should facilitate investigations designed to develop an understanding of the heterogeneity and pathophysiology of acute leukemias. The intention here is to continue studies characterizing leukemic cells, their heterogencity and state of differentiation with heteroantibodies, antibodies and monoclonal antibodies defined by somatic cell hybridization techniques. The identification of these unique antigens by serologic methods is the first step in their isolation for biochemical characterization.

It is believed that these studies will provide insight into the nature of the leukemic process and the relationship between these antigens and those found during normal cellular differentiation. These antibodies, including T, T3, T4, T5, T6, T9, T10, Ia, and J-5, have already allowed the initial classification of T and Null cell leukemias and determination of their relation to normal differentiation.

225. HUMAN LEUKEMIA-ASSOCIATED ANTIGENS

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The long-term goal of this research project is to isolate and characterize the various membrane antigens associated with human leukemia and lymphoma cells. Since leukemia and lymphoma cells usually represent monoclonal proliferations of various lymphoid and myeloid series cells, antisera elicited to these malignant cells will also be studied for their ability to define normal cell antigens. We will also attempt to devise and improve our present methods for the detection of leukemia and lymphoma cells and to classify these diseases more precisely. Emphasis will be placed on those antigens which will define subpopulations of lymphoid or myeloid cells and on antigens which may distinguish certain stages of differentiation of these cell types.

226. IMMUNOCOMPETENT CELLS IN ACUTE LYMPHOCYTIC LEUKEMIA

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The specific aims of this proposal are: 1) To determine if phenotypically distinct ALL blasts possess cell surface antigens which are also expressed by a cell subset of normal regenerating bone marrow (a putative stem cell population) and to compare these antigens with those on normal lymphoid cells. Cells from regenerating bone marrow will be obtained from patients in remission after anticancer treatment has been stopped. These bone marrow cells and cell subsets will be characterized by their membrane markers, surface antigens and functional properties. 2) To establish if cell surface phenotypes of normal lymphocytes, normal lymphoblasts and neoplastic lymphoid cells correlate with the biochemical and pharmacological characteristics of these cells. 3) To determine in children with ALL the kinetics of recovery of T lymphocyte subsets and their helper or suppressor role upon B cell function after cessation of therapy.

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227. MYELOGENOUS LEUKEMIA-ASSOCIATED ANTIGENS

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We have developed and characterized the only myelogenous leukemia cell line (K-562) available in culture, originally derived from a patient with CML. The K-562 has the Philadelphia (Ph1 plus) chromosome after 8 years in culture. It has been extensively examined and found to be free of virus and mycoplasma; it does not have T- or B-cell markers but does have Fc receptors. These cells, when injected into rabbits, monkey, or goat, elicit the production of specific antibody which demonstrates complement-mediated cytotoxicity for K-562 and leukemic cells of patients with leukemia. Specificity for myelogenous leukemia cells is obtained after absorption of antisera with normal peripheral leukocytes and bone marrow cells as well as with leukemia cells of others types (i.e., AML, ALL, CLL). The purpose of this project is: 1) to refine our fractionation procedures to isolate the target antigen of K-562 cells recognized by goat monospecific immunoglobulins; 2) to determine definitively if the target antigen reactive with antibody is the same as, or different from, that recognized by human natural killer cells; 3) to assess the presence of immune complexes in the sera of patients with leukemia and to characterize the components of such complexes; 4) to study the potential for differentiation of K-562 cells with known inducers of murine leukemia cells such as dimethylsulfoxide and sodium butyrate; and 5) to use the immunologic reagents (monospecific globulins and characterized antigens) as reference standards to accurately detect incomplete remission and early relapse in patients with leukemia.

228. CELL SURFACE ANTIGENS IN MURINE LEUKEMIA

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The aim of the proposed project is to investigate the biochemical makeup of H-2 antigens and their chemical and/or structral relationship to viral antigens on murine leukemia cells. Since it has been demonstrated that there are interactions between H-2 antigens and virus and that T-cell mediated killing of virus infected and transformed cells is H-2 restricted, a concerted effort will be made to elucidate the biochemical nature of H-2-virus interactions. Monoconal anti-H-2 antibody will be produced in order to circumvent problems due to contaminating antibody so frequently found in conventional anti-H-2 antisera. This antibody as well as highly specific antibody against viral antigens will be used to characterize the antigenic profile of virus infected and transformed cells. In addition these antibodies will be used to isolate H-2 and viral components from cell extracts via immunoprecipitation. The isolated antigens will be extensively characterized using biochemical micromethodology and their relationship with one another as well as their relationship to their counterparts on uninfected or untransformed cells established. Finally an effort will be made to determine whether chemical and/or structural alterations in the expression of H-2 antigens on leukemic cells correlate with the progress of the disease or the immune competence of the host. The information thus obtained should contribute significantly to our understanding of the role of H-2 antigens in tumor cell physiology and in host response to malignant disease and will make possible an accurate assessment of the potential of such cell surface alterations for immunodiagnosis and immunotherapy.

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229. CLASSIFICATION OF HUMAN LEUKEMIAS BY QUANTITATION OF TUMOR ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

230. LEUKEMIA-SPECIFIC ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

231. HUMAN DIFFERENTIATION ANTIGENS AND SOMATIC CELL HYBRIDS

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We have continued to produce monoclonal antisera to human cell surface antigens by the technique of somatic cell hybridization. An antiserum (anti-HLe-1) recognizing a common leukocyte antigen has been most extensively analyzed. Only cells of the lymphoid, myeloid and erythroid series bear the antigen, but while mature lymphocytes, monocytes and granulocytes retain it, it is lost during maturation of erythrocytes and platelets. Fractionation of bone marrow by fluorescence-activat-

ed cell sorting reveals that CFU-C are weakly positive. Further enrichment of myeloid precursor cells by removal of mature myeloid cells from the weakly stained population should be possible by density gradient fractionation. The antibody also shows promise as a diagnostic reagent, particularly in distinguishing lymphoid from non-lymphoid tumors in tissue sections.

A number of other surface markers, including two that are restricted to mature granulocytes, are under investigation. We have detected, though not yet cloned, antibodies which distinguish brain tumor-derived cell lines from other cell types.

We have also made human T by human T cell hybrids using azaguanine-resistant T-ALL-derived lines as the immortal partner. The properties of these lines are under investigation.

232. GENETIC AND BIOCHEMICAL ANALYSIS OF HUMAN LYMPHOID CELL SURFACE ANTIGENS

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The genetic and biochemical analysis of human cell surface antigens has been made possible by the techniques of somatic cell hybridization. The dominant expression of human cell surface structures on human x mouse cell hybrids has allowed the mapping of human surface antigens to specific human chromosomes. More recently, the ability to generate, using the method of Milstein, monoclonal antibodies to cell surface structures has facilitated their biochemical characterization.

We propose to begin a genetic and biochemical study of human lymphoid cell surface antigens. Xenogeneic (cross-species) monoclonal antibodies to human lymphoid cells will be generated and screened by an indirect binding assay. Standard antibody fluorescent techniques will be used in conjunction with a fluorescence-activated cell sorter to separate antigen positive cells in human x mouse cell hybrids. Karyotypes of these cells will be examined to correlate specific human chromosomes with antigen expression. Through biochemical labelling techniques followed by monoclonal antibody immunoprecipitation, these antigens will also be characterized as to molecular weight and subunit structure.

The study of cell surface antigens of human lymphoid cells may help define functionally and developmentally distinct subsets of lymphoid cells. This information may lead to the clinical management of immunological diseases and neoplastic progression. Moreover, the mapping of the genes determining these cell surface structures will contribute to our knowledge of the organization of human chromosomes.

233. MALIGNANT BEHAVIOR AND CELLULAR ANTIGEN EXPRESSION

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Cytogenetic changes and malignancy. We shall continue to explore the role of specific cytogenetic changes in murine and human lymphomas and leukemias, with particular focus on the occurrence and physiological role of chromosome 15 trisomy in the murine T-lymphoma and/or the 8:14 translocation in the human B lymphoma system.

Somatic hybridization in vitro. Continued studies will focus on the expression of surface markers, including genetically determined, differentiation related, virally induced and tumor associated markers. The information on the tumor associated markers may be helpful for a more rational design of 'xenogenization experiments'. For the EBV-system, the hybrid studies may decisively answer the important question whether the specific chromosomal change or the viral genome are essential for the maintenance of in vivo tumorigenicity.

Continued studies of host-tumor cell fusion in vivo. We shall explore the kinetics of in vivo fusion, and perform marker studies that may be helpful in deciding which host cell types are involved. We shall try to utilize the system for the in vivo production of hybridomas and T/T cell hybrids. As a further step in our analysis of malignancy by somatic hybridization, we shall test the tumorigenicity of tumor-host cell hybrids that were generated after short term in less than 24 hours after inoculation.

Development of new variant and mutant lines. By the use of our recently developed column selection method we shall generate immunoresistant sublines with reduced antigenicity and an increased ability to survive in face of host rejection. This approach also permits a study of the question whether the serologically defined, i.e. antibody binding surface sites are identical with the targets of cell mediated rejection.

Genetic control of the immune response. This study focuses on the dissection of F1 hybrid resistance patterns against different tumors. We shall analyse the H-2 linked localization of resistance, in relation to the linkage relationships already demonstrated. We shall also explore the suggested connection between in vivo hybrid resistance and in vitro natural killer (NK) cell activity.

234. LYMPHOCYTE CELL SURFACE ANTIGENS, NORMAL AND NEOPLASTIC

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The biologic, functional and chemical characterization of human lymphoid cell surface antigens controlled by genes of the major histocompatibility complex (MHC) is under investigation. HLA typing of disease groups and families wherein several members are diseased has demonstrated a complex association of the MHC with the disease condition. Antigens not found in linkage disequilibrium in a normal population are found to mark psoriasis and psoriatic arthritis. Connective tissue diseases with overlapping clinical manifestations have distinct distribution of HLA alloantigens suggesting specific genetic contributions of the MHC to the disease state. Alloantisera detecting murine Ia antigens cross-react with human B lymphocyte and monocyte antigens (HLA-Dr). Human alloantisera detecting HLA-DRw antigens precipitate combinations of molecules of 4 different molecular weights. These antisera, when reacted with monocytes, induce specific T cell suppression of immunoglobulin synthesis and antigen stimulation. Monoclonal antibodies to lymphocyte cell surface antigens appear to identify functional subsets of T lymphocytes.

BIBLIOGRAPHIC REFERENCES: Mann, D. L., Kaufman, J., Orr, H., Robb, R., and Strominger, J.: Serologic and structural studies of DR-related antigens. Transplant. Proc. 11: 668-673, 1979. Mann, D. L. and Sharrow, S. O.: B-cell alloantigens on T lymphocytes. Transplant. Proc. 11: 1774-1779, 1979.

235. H-2 ANTIGEN EXPRESSION IN CULTURED MOUSE LEUKEMIA CELLS

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The aim of the proposed research is to study the regulation of expression of the H-2 antigens. The approach will be to isolate, from heterozygous mouse leukemia cells in culture, variants that have undergone stable heritable alterations in the expression of the H-2 antigens and to use these variants to investigate the normal controls of H-2 antigen expression. Variants will be isolated by two in vitro techniques. One of these, anti-H-2 antibodies in the presence of complement, has been used successfully in the past two years to isolate several interesting variants. More variants will be sought using this method. In addition, H-2 directed cytotoxic T-cells will also be used as selective agents.

Once isolated, the variants will be studied by genetic techniques such as karyotyping and somatic cell hybridization; biochemical procedures such as gel-electrophoresis and, in collaboration with Dr. S G. Nathenson, peptide mapping, where warranted; and by immunological methods such as radioimmunoassay, serological responses on inoculation into the syngeneic animal, and interactions with T-lymphocytes, immunized against wild type antigens.

The rates of emergence of these variants will be determined by the fluctuation analysis. This experiment will also be used to determine whether the variants preexist the selection, or are in some way induced by the selective agents. The effect of various mutagens on the rates of emergence will be studied.

Recently, much attention has been focused on the role of the H-2 antigens in the immune response to viral antigens. The

H-2 variants and the wild type cells form an isogenic system where this role of the H-2 antigens can be studied. The immune responses of syngeneic animals to the Friend virus associated antigens will be investigated. Some preliminary experiments have suggested that certain combinations of H-2 antigens may interfere with or augment the T-cell response to the antigens associated with the Friend virus.

236. STUDY OF GROUP 5 ANTIGENS IN HEMATOLOGIC MALIGNANCIES

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The leukocyte group 5 antigenic system has two dominant alleles (5a and 5b) which segregate independently of HLA. We have discovered that the 5a allele of this system has a striking association with acute lymphoblastic leukemia (ALL), the strongest association yet found of an antigen with human leukemia. In subsequent studies, the 5a allele was also found to be associated with chronic lymphocytic leukemia and lymphocytic lymphoma but not with histiocytic lymphoma nor with Hodgkin's disease. We propose, by use of the leukocyte agglutination and antibody-dependent cell-mediated cytotoxicity assays, to 1) study the association of 5a with ALL in an attempt to gain insight into the cause of ALL; 2) determine the significance of the 5a antigen as a prognostic marker; 3) study patients with other hematologic disorders for increased frequencies of the 5a and 5b alleles; and 4) attempt to assign the group 5 locus to a particular linkage group.

BIBLIOGRAPHIC REFERENCES: Association between leukocyte group 5a antigen and lymphoid malignancy. R.P. Warren, R. Storb, D.D. Nguyen, and E.D. Thomas. Blood 54: No. 5 Suppl. 214a, 1979.

237. PROGRAM PROJECT IN TUMOR AND ALLOGRAFT IMMUNITY

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This program project represents a multidisciplinary approach to problems in allograft and tumor immunity; it attempts to approach the problems of antigen recognition and immune response to normal histocompatibility and tumor antigens by studying the genetic control of alloantigens, the nature of the alloantigens and tumor antigens as components of the lymphocyte or tumor cell membrane, the cellular response to these various antigenic stimuli, and the physiological behavior of the membrane antigens as it relates to the immune response to the cells carrying the antigen. The program is composed of two research parts which are intimately interdependent and complementary: one, genetic and biological studies, and two, biochemical studies of lymphocyte and tumor cell membranes, both in relation to allograft and tumor immunity.

The first part is concerned with a study of the genetic control of normal histocompatibility antigens, primarily those controlled by the major histocompatibility complex (MHC), at both the stuctural and functional level. The MHC will be used as a model for tumor associated antigens and attempts will be made to extrapolate use of the in vitro models developed for the allograft response to the study of tumor associated antigens. The second part envisages a detailed biochemical study of the membranes of normal lymphocytes and leukemic cells. The purpose of such a study is primarily not to understand the chemistry of membranes and membrane components but rather to learn more about the genetic control of lymphocyte membrane structure and its relation to immune response, leukemia-associated membrane changes, and the fundamental mechanisms underlying immune response to leukemia and other tumors.

238. CHARACTERIZATION OF HLA ANTIGENS OF DONOR'S LYMPHOCYTES

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No summary has been provided to the Smithsonian Science Information Exchange.

239. DIFFERENTIATION ANTIGENS ON HUMAN LYMPHOCYTES

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No summary has been provided to the Smithsonian Science Information Exchange.

240. CHARACTERIZATION OF NEW HUMAN IA AND LEUKEMIA ANTIGEN

Mohanakumar, T., Virginia Commonwealth University, Medical College of Virginia, Dept. of Surgery, *1200 E. Broad St., Richmond, Virginia,* 23298, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

241. HISTOCOMPATIBILITY COMPLEX AND THE EXPRESSION OF ANTIGEN SYSTEM IN LEUKEMIA

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This is part of a broader project. A summary of this subproject is not available.

242. STUDIES ON SERUM ANTIGENS (AND ANTIBODIES) ASSOCIATED WITH MALIGNANT DISORDERS

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OBJECTIVE: To identify and characterize serum components associated with malignant disorders, to compare different oncofetal components and to assess the usefulness of their demonstration, alone or in combination, in diagnosis, prognosis or monitoring of treatment.

APPROACH: Antisera against human serum leukemia associated antigen (LAA) are produced in animals, and used to detect the presence of LAA in serum samples from different patient populations and to determine LAA quantitatively by the 'rocket' immunoelectrophoretic technique. The findings obtained are compared to clinical diagnosis and stage of disorder to assess the practical usefulness of qualitative or quantitative LAA determination. The simultaneous determination of several other oncofetal components is conducted in order to reveal any relationship between such components and LAA. The spectrum of components examined includes ferritin, alpha-fetoprotein, carcinoembyronic antigen (CEA) and 'tissue peptide antigen' (TPA). Furthermore, several oncofetal components are subjected to in-depth biochemical analyses to reveal any biochemical relationships. The site of synthesis and the mechanisms regulating these components are studied in different ways, including analyses of various tissues and body fluids with respect to these components.

PROGRESS: LAA has been demonstrated to be an oncofetal component, strongly associated with malignant blood disorders. LAA status at the time of diagnosis has predictive value, and a change from negative to positive LAA test in a leukemia patient in remission is a warning that a relapse may occur within several weeks or a few months. LAA occurs much less frequently in patients with various other tumors and only very rarely in normal sera. Intact LAA in whole serum is a high molecular weight protein (molecular weight above 200,000), but an apparent subunit with a molecular weight of 40-60,000 has been isolated. LAA is to a great extent independent of most other oncofetal components, including TPA. TPA, like the other components, is present in normal amniotic fluid and in fetal liver, proving its oncofetal characteristics. Several reports on LAA have published (Scand. J. Haematol. 18, 13-19 (1977); ibid 19, 362-469 (1977); Proc. 25th Coll. Prot. Biol. Fluids, 755-761 (Pergamon Press 1978); Scand. J. Immunol. 8, suppl. 8, 641-645 (1978)).

243. HUMAN LEUKEMIA ASSOCIATED ANTIGENS

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The main objective of the proposed research is to develop methods of defining and isolating cell surface neoantigens associated with different subclasses of human leukemias and to employ them for immunodiagnosis and monitoring. Several well-characterized hetero- and monoclonal antibodies defining

leukemia-associated antigens will be evaluated for their usefulness in early detection of antigenic changes by microcytotoxicity, immunofluorescence and flow microfluorometric analysis using the TPS-1 cell sorter. The antigens isolated by immunoprecipitation with heteroantisera will be used for the production of additional monoclonal antibodies defining leukemic cell surface antigens. Another aspect of this study will be immunofluorescent identification of peanut agglutinin receptors on cells from acute lymphoblastic leukemias and evaluation of their suggested prognostic significance. Normal and leukemic cell surface molecules reactive to peanut agglutinin will also be isolated by affinity chromatography, with special interest directed towards the possible characterization of new leukemia-specific components.

244. PRODUCTION AND USES OF MONOCLONAL ANTIBODY PROBES SPECIFIC FOR HUMAN LEUKEMIA CELL ANTIGENS

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The aim of this project is to produce a series of monoclonal antibodies capable of distinguishing antigens from a variety of classified leukemias and thereby to allow their use clinically to classify undifferentiated leukemias, and follow their course of remission and relapse during therapy.

These antibodies will also be used in more fundamental, long-term studies of human leukemia. They will be used as probes to investigate the relationship between leukemia-specific antigens and normal cell antigens and to complement and extend the study, now in progress at the institute, of the differences in the messenger RNA populations between different types of leukemia cells.

The monoclonal antibodies will also be used to identify recombinant clones carrying leukemia-specific sequences which give rise to leukemia-specific antigens. These cloned genes can then be used to study the expression of individual genes in normal and leukemic cells to answer a number of important questions regarding their regulation (or lack of it).

The great advantage of the use of leukemic cells for these studies is that of readily available human tumors these are the most clearly defined, in clinical terms.

245. HETEROANTISERA TO LEUKEMIA-ASSOCIATED ANTIGENS

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OBJECTIVES AND APPROACH: 1) To produce antisera to human leukemia-associated antigens (LAA) with specificity for morphologically characterized leukemic blast cells. Assays using antibody derivatized beads and the fluorescence activated cell sorter will be used to define reactivity of antisera towards specific cells and to distinguish the reactivity of leukemic blast cells from that of myeloblasts in non-leukemic bone marrow.

2) To characterize the nature of human acute leukemia-associated antigens by immunological means. The distinction of LAA from HL-A and B-cell alloantigens, cell cycle specific antigens, differentiation antigens, and viral antigens and reaction with non-leukemic stem cells will be studied. Change in LAA from presentation of acute myeloblastic leukemia to subsequent relapse will be investigated.

3) To characterize the biochemical nature of human acute leukemia-associated antigens and the biological role of the carbohydrate moieties of leukemic cell surface determinants. Soluble labeled cell surface antigen will be isolated and purified using coprecipitation with antibody to LAA to monitor activity. The role of surface carbohydrates will be assessed by inhibition of antisera with oligosaccharides, differential lectin binding, radioimmunoassay utilizing binding of trace-labeled lectin to derivatized beads and membrane oligosaccharide regeneration following enzyme stripping.

4) To develop diagnostic criteria for determining total body burden of leukemic blast cells. Circulating soluble LAA in serial samples of patient's serum will be quantitatively assessed by radioimmunoassay utilizing inhibition of coprecipitation. Serial bone marrow samples will be assessed for numbers of immunoreactive cells utilizing antileukemic hetero- and allosera.

246. LEUKEMIA ASSOCIATED ANTIGENS

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We have raised in rabbits a serum to human common acute lymphocytic leukemia cells (cALL). Following absorption with B cells, the serum is cytotoxic for cALL cells but not for normal B or T lymphocytes. The serum reacts with a cALL antigen of 98,000 daltons. We also have produced a hybridoma cell line which secretes a cytotoxic monoclonal antibody of the same specificity. We have also produced a cytotoxic monoclonal antibody against an acute myelocytic leukemia (AML) antigen. The antibody does not react with normal peripheral blood white cells, but it does react with myeloid bone marrow stem cells.

We have developed a panel of heteroantisera that can subclassify the type of leukemia into diagnostically meaningful subtypes. The antisera react with the following antigens: Ia-like, T cell, blast cell, cALL and AML.

We have raised heteroantisera to a subpopulation of peripheral blood T lymphocytes and they react with two different cell membrane antigens.

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247. PRODUCTION OF MONOSPECIFIC LYMPHOMA ANTIBODIES

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OBJECTIVES: Attempt to produce xenogeneic monospecific antibodies to human lymphoma and leukema associated antigens. Attempt to fuse antibody producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibody against tumor-associated antigens in vitro. Compare two hybrid techniques. One method to be compared will involve the hybridization of preselected clones using a method developed by Klinman. The second method will involve hybridization of immune spleen cells from whole spleens of immunized mice, then cloning the resulting hybrids, and finally searching for monospecific antibody. Also, evaluate the specificity of the presumed monoclonal antibodies and investigate the pattern of antigenic tumor-specific determinants on malignant lymphoid cells.

248. LEUKEMIA AND NORMAL LEUKOCYTE-ASSOCIATED ANTIGENS

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We propose to detect antigens associated with normal and malignant hematopoietic cells and solid tumor cells by 1) raising heteroantiserum in rabbits and 2) by the hybridoma technique. We plan to determine the molecular characteristics of the antigens and to utilize these antiserum reagents in the diagnosis and treatment of these diseases. We will also utilize these reagents to better understand the antigenic characteristics of hematopoietic and tumor stem cells. Antisera will be screened on panels of normal and malignant cells by cytotoxicity, indirect immunofluorescence and 125I Staph. aureus protein A. Characterization of these antigens will be by immunoprecipitation and run on SDS polyacrylamide gels. Stem cell antigens will be studied by cytotoxicity against human bone marrow in vitro cultures (myeloid colony forming units or CFU-C).

We have reported antiserum and specificity for Null acute lymphocytic leukemia (ALL), acute myelogenous leukemia, and T-ALL cell lines. We have recently reported on the antigenic makeup of CFU-C and its utility in bone marrow transplantation. We are currently studying a human granulocyte antiserum, an antiserum to MOLT-4, a T-ALL cell line, that detects three separate T-associated antigens, and an antiserum to K562 chronic myelogenous leukemia cell line.

249. NUCLEAR ANTIGENS AS MARKERS IN HEMATOPOIESIS

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In recent years immune sera have been raised which frequently recognize nuclear antigens with limited cellular distributions. Immunogen prepared from isolated peripheral blood human granulocyte chromatin was used to produce antisera specific for human neutrophilic granulocyte nuclear antigens. We propose the following areas for investigation of these unusually specific nuclear antigens: (1) The antigens will be isolated and characterized. Their localization within the chromatin structure will be determined using recently developed enzyme digestion and fractionation schemes. This information combined with the molecular characteristics will be used to assign functional roles. (2) Immunocytochemical staining of normal and pathologic bone marrow aspirates and peripheral blood specimens shows that all late stage myeloid cell nuclei react. To further elucidate a possible role in cellular differentiation, the antigens will be studied in cultures of myeloid leukemic cells, both induced and non-induced to differentiate. In another approach, antigens will be studied in cultured cells as well as specimens of myeloid leukemia separated into early and late stages by density gradient centrifugation. (3) The normal myeloid chromatin antigens were found in chromatin isolated from a specimen of CML. Immunocytochemical staining of CML and AML showed nearly every cell nucleus reacting positively. However, chromatin isolated from a cell culture of acute promyelocytic leukemia showed only one of the three normal myeloid chromatin antigens by immunodiffusion reaction. No cross reacting antigens have been found in any other leukemias. The usefulness of the antisera in differentiating types of leukemias or in monitoring therapy will be determined by comparing the immunocytochemical reaction with the results of current diagnostic methods. (4) New antisera will be developed to chromatin antigens of other hematopoietic cell types, both normal and leukemic.

250. DIFFERENTIATION ANTIGENS OF HUMAN LYMPHOBLASTOID CELLS

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The goal of this work is to develop antibodies to differentiation antigens found on cultured human lymphoblastoid cells. Three cell lines, derived from two patients with acute lymphoblastic leukemia (ALL), were chosen as immunogens. Two of the lines, RPMI 8392 and RPMI 8402, were derived from the same patient and are, therefore, autochthonous cell lines. RPMI 8402 cells are T lymphoblasts that emerged from the leukemic stemline, while RPMI 8392 cells are immunoglobulin-producing, Epstein-Barr viral genome-containing B lymphoblasts that arose from the patient's residual normal B cells. The third cell line, NALL-1, arose from the blood of a 14 year old boy with non-T, non-B cell ALL. These cells produce terminal deoxynucleotidyl transferase (TdT) and bear both the Ia and common ALL antigens. Cultured NALL-1 cells do not produce immunoglobulin nor do they form E rosettes. Thus, NALL-1 cells are phenotypically very similar to 'common' ALL cells. The purpose of our project is to generate antibodies to T, B, and lymphoid stem/progenitor cell surface differentiation antigens that are found on these cells.

Our plans include the generation of antisera in rabbits to each cell line. We seek the class of antibodies that binds only to one of the two cell lines. These antisera are being analyzed to define specificities against a range of differentiation antigens found on mature, differentiating and neoplastic lymphoid and hematopoietic cells.

251. ANTISERA TO HUMAN LEUKEMIA-ASSOCIATED ANTIGENS

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Our continuing goal is to assess the usefulness of antisera prepared against surface antigens of leukemic cells in the clinical diagnosis and management of human acute and chronic myeloid leukemia.

Studies will emphasize the preparation of purified soluble antigenic material from cultured human leukemic myeloblasts, and the development of specific antisera by using primate and murine antisera and employing in vivo and in vitro techniques.

The overall intent is to develop immunofluorescent assays which will be useful in delineating clones of leukemic cells in remission patients, and also to develop reagents for specific radioimmunoassay of leukemia-associated antigen in human serum.

B. THYMUS-LEUKEMIA ANTIGENS

252. THY-1 ANTIGEN - LATENT ANTIGEN ALLOWS RAPID EXPRESSION ON THE CELL SURFACE DURING DIFFERENTIATION

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Thy-1 differentiation antigen is a glycoprotein (molecular weight approximately 18,000) found on cell surfaces in various tissues (lymphoid, hemopoietic, nervous and mammary) at certain stages of their differentiation. Antigenic determinants are on the protein component of the molecule and are of two main types: xeno and allo, recognized by xeno- and allo-antisera, respectively. Both are present on a single molecule.

Enriched populations of thymocyte precursors (prothymocytes) have been obtained by BSA density gradient centrifugation. These cells have some partially exposed Thy-1-molecules (xeno determinants only) at the cell surface (anti-xeno antibodies bind to the cells, anti-alloantibodies do not). Additional Thy-1 is totally hidden (unavailable to antibody) but is uncovered by detergent solubilization. Moreover, treatment of prothymocytes with dibutyryl cyclic AMP, which mimics in vivo induction with thymic hormones, leads to the rapid (20 min.-3 hours) exposure of allo-antigenic determinants typical of mouse thymocytes. The function of such 'latent' antigen may be to provide rapid changes in the cell surface during differentiation, thus avoiding delay involved in de novo synthesis and membrane insertion of surface molecules.

'Latent' Thy-1 may also be involved in a second system. Unactivated rat platelets are Thy-1-negative. However, very early in activation Thy-1 becomes rapidly exposed on the platelet surface. This exposure may be due to platelet shape change whereby Thy-1 that was concealed within canaliculi (surface invaginations) is uncovered as these evert.

253. PURIFICATION AND STRUCTURAL CHARACTERIZATION OF GLYCOPROTEINS RELATED TO THY-1

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Thy 1:1 and Thy 1:2 are differentiation alloantigens expressed on certain rat and mouse cells. We have purified low molecular weight, integral, plasma membrane glycoproteins which carry these alloantigens by a procedure which involves chloroform-methanol delipidation of homogenized mouse brain, detergent extraction in sodium deoxycholate and affinity chromatography on lentil-lectin sepharose. Pure Thy-1 glycoproteins were isolated from the lectin-bound fractions by successive gel filtration on Sephacryl S-200 columns equilibrated in sodium deoxycholate or urea/NP40. The purified molecules from both AKR(Thy 1:1) and CBA(Thy 1:2) brain run as doublets on polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS PAGE), with apparent molecular weights of 25,100 and 25,300 (AKR) and 25,800 and 25,400 (CBA). Amino acid composition data suggest that there are only minor amino acid differences between the two allotypes.

Rabbit antisera raised against purified Thy-1 glycoproteins from rat or mouse recognize additional species-specific and species-cross-reactive determinants on the molecule. We have used the species cross-reactive component of such antisera to identify homologous glycoproteins in human brain. The human Thy-1 homologue also runs as a doublet on SDS-PAGE, with apparent molecular weights of 26,400 and 25,800, and has a remarkably similar amino acid composition to that of rat and mouse

Thy-1. Following reduction, alkylation and succinylation the molecule can be efficiently cleaved with trypsin and Staphylococcus aureus V8 protease and the peptides isolated by high pressure liquid chromatography. The amino acid compositions of these peptides have been compared with those of similar peptides derived from rat brain Thy-1. Such comparisons indicate that the Thy-1 glycoprotein is strongly conserved between species.

254. EXTRACELLULAR THY-1 IN THE RAT - A POSSIBLE ADHESIVE MOLECULE

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Immunofluorescence and silver impregnation studies of wax-embedded tissue sections have shown Thy-1 antigen to be associated with certain collagen-based interstitial and vascular connective tissue in the rat. This association is highly selective, but does not correlate with any known collagen type, blood vessel type or permeability. Thy-1-positive connective tissues in lymphoid organs are: lamina propria of the small intestine, dermis of the skin, vascular basement membrane of lymph node post-capillary venules, and central arteriole of splenic white pulp. These areas and blood vessels are all involved in the recirculation and homing of mature T and B lymphocytes (both are Thy-1-negative in the rat). Thus Thy-1 may function as an adhesive molecule, mediating selective lymphocyte migration.

255. MOLECULAR ASPECTS OF THE CELL SURFACE EXPRESSION OF TL

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ASL-1w immune leukemia cells grown in the presence of anti-thymus leukemia antigen (TL) sera have a greatly reduced growth rate when compared with control cultures grown in the presence of anti-H-2 or normal mouse sera. The kinetics of the decline in DNA synthesis and kinetics of accumulation of cells in metaphase in cultures treated concomitantly with colcemid suggest that anti-TL treatment blocks the cells in G-2 phase of the cell cycle. The hypothesis of this proposal is that the anti-TL induced growth inhibition of ASL-1w can serve as a model system to aid in understanding growth regulation as modulated by membrane events.

Experiments which will be done include 1) confirmation of the cell cycle phase of the anti-TL block, 2) cell cycle phase of TL biosynthesis and degradation, 3) kinetics of protein and RNA synthesis prior to and after release of the anti-TL block.

256. NEOPLASTIC AND NORMAL CELL THYMUS-LEUKEMIA ANTIGENS

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The major goals of this research project are (1) to study the protein structure of various murine thymus-leukemia antigens (TLA), (2) to analyze the differentiation pathways and immune functional properties of T cells that are derived from TLA-positive or TLA-negative thymocytes (of TLA positive mouse strains), and (3) to prepare murine fused cell hybrids and clone anti-TLA secreting hybridomas. These studies will attempt to compare and contrast the major structural and biological properties of TLA with the antigens of the major histocompatibility complex (MHC). The principal methods used in these studies will be radioisotope labeling of cell membrane antigens, immunospecific isolation and polyacrylamide gel electrophoresis of labeled TLA and MHC antigens. Proteolytic cleavage, acid cleavage and peptide mapping will also be used to characterize and compare the TLA associated with different TLa haplotypes. Thymocyte transfers between TLA-congenic mouse strains will be used to study the differentiative potential of TLA-positive and TLA-negative thymocytes. TLA and MHC antigens are coded for by linked genes on the seventeenth chromosome. Their gross chemical structure is very similar and it is probable that each arose from a common ancestral gene. This genetic and protein structural evidence forms the basis for the assumption that these two classes of lymphocyte surface antigens have similar and related functions.

257. BIOLOGICAL ROLE OF ALLOANTIGENS

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The purpose of this investigation is to study the role of murine Thy-1 cell surface alloantigens in T-lymphocyte and neuronal differentiation. Utilizing aggregation cultures of embryonic tissue the ability of anti-Thy-1 serum as well as purified Thy-1 to block cell sorting events will be determined. Providing the reaggregation of embryonic thymus and brain tissue can be inhibited by this method, Thy-1 will be purified and that portion of the molecule responsible for the recognition phenomena deciphered. Other 'differentiation alloantigens' expressed on lymphoid and nervous tissue will also be investigated for their role in the differentiation of these tissues. Moreover, heteroantisera against precise regions of nerve endings (synaptosomes) will be raised and investigated in the cell aggregation system in an attempt to define other cell surface components which may be involved in synaptogenesis. These antisera will be useful in purifying select membrane components involved in differentiation. The concepts derived from this study should be most useful in terms of understanding differentiation in general, whether normal or abnormal, and of envisioning ways of directing cell sorting events. Knowledge concerning normal cell surface components which govern cell sorting would be of obvious significance in understanding cell surface aberrations as a consequence of malignant transformation.

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258. GENETIC BASIS OF ANTIGENIC VARIATION

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The long-term objective of this project is to understand the genetic and epigenetic mechanisms by which the tumor cell regulates the expression of its tumor-associated antigens. Antigenic variants of murine lymphomas growing in tissue culture are being used to study the genetics of several model normal and tumor-associated cell surface antigens. Particular emphasis is being placed on understanding the Thy-1, T200 and TL glycoproteins. Mutant cell lines which do not express these glycoproteins on their cell surface are being isolated by cytotoxic immunoselection and characterized using a combination of somatic genetic and biochemical approaches. Hybrids between cell lines expressing Thy-1 and cell lines representing cell types which normally do not express Thy-1 are also under study. By this means the number and nature of the genes involved in the biosynthesis of a particular cell surface molecule are being defined.

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259. IMMUNOGENETIC MAPPING OF THE CELL SURFACE

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The objectives of this grant proposal are (1) to map, using serological techniques, the topographical arrangement of alloantigens on the cell surface of the mouse thymocyte and peripheral T lymphocyte, and (2) to investigate the induced rearrangement of these alloantigens called supramolecular 'repatterning' which is triggered by binding of an appropriate antibody to an alloantigenic site or, in the case of H-Y, by the hormone testosterone. We will initially study the physical interrelationships

between the mouse alloantigens H-2D, H-2K, H-2L, Lyt-1, Lyt-2, Ly-5, TL, Thy-1, and Qa-2, and determine whether the relative positions of these cell surface components can be altered by binding with antibody. We will also determine whether such agents as colchicine and Cytochalasin B will inhibit alloantigenic repatterning. Finally, we will determine whether other agents besides antibody and testosterone will induce specific cell surface repatterning. Some genetic studies will also be performed. Methods to be used include the dual blocking assay, radio- or enzyme-immunoassays, antiserum production, hybridoma formation, cytotoxicity testing, and absorption procedures.

260. CONTROL OF CELL DIVISION IN LEUKEMIA CELLS

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Previous studies have shown that the cell surface expression of thymus leukemia antigen (TL) on ASL-1w leukemia cells varies with the progression of the cells through the growth cycle. Expression of TL is maximal in S phase and its quantitative expression varies directly with DNA synthesis. In the present study the effect of anti-TL serum on the growth of ASL-1w cells was examined. The antiserum, tested in the absence of complement, affected the growth of these cells in biphasic manner. When the antiserum concentration was 0.1% or greater, there was a rapid decline in the rate of DNA synthesis and, after 5-7 hours, cell death occurred. When the antiserum concentration was 0.067% or less, the decline in the rate of synthesis of DNA did not become apparent until 5-6 hours after treatment. Under these conditions there was approximately a 20% increase in cell number after 24 hours of culture. The hypothesis that treatment of ASL-1w cells with the lesser concentration of anti-TL serum blocks the cells in G-2 phase of the cell cycle is discussed. The objective of this proposal is to test this hypothesis. Relevant experiments which will be carried out are: 1) Biosynthesis of TL and relationship of cell cycle to regulation. 2) Use of various cell cycle specific drugs to determine their effect on the regulation of biosynthesis of TL. 3) Use of synchronous cell cultures to study the relationship between TL and regulation of cell division.

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261. THY-1 DIFFERENTIATION ANTIGEN IN RAT MAMMARY TISSUE

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We have demonstrated the presence of Thy-1 antigen on in vivo rat mammary tissue, using immunofluorescence on wax-embedded tissue sections. Thy-1 is found on ductal and alveolar myoepithelial cells of normal, pregnant and lactating animals (myoepithelial cells identified by Coomassie blue staining of myofilaments and by their position between the epithelial cells and the basement membrane). Thy-1 positive myoepithelial cells were also found in Dimethylbenzathracene and N-methylnitrosourea-induced mammary adenocarcinomas, and in adenocarcinomas derived from Rama 25 cells (cuboidal epithelial stem cell line).

Rat mammary cell lines were also studied by immunofluorescence. Rama 25, a stem cell line, was Thy-1 negative, whilst its myoepithelial derivative (Rama 29) was Thy-1 positive. Thy-1 antigen was also present on the elongated cells that appeared as fresh converts within several stem cell line cultures (Rama 75, 401 and 402). Clonal cell lines intermediate in morphology between the stem cell cuboidal line Rama 25 and the elongated myoepithelial-like cells of Rama 29 have been isolated. These intermediate types convert to myoepithelial-like cells when cultured at high frequency; during this conversion the appearance of surface Thy-1 correlates with the main morphological change and with the appearance of the extracellular matrix protein, fibronectin. Whether Thy-1 plays a causative role in the conversion process remains to be established.

262. ACUTE LYMPHOBLASTIC LEUKEMIA - AN IMMUNOLOGICAL STUDY OF A POSSIBLE T-CELL MALIGNANCY

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The application of T- and B-cell markers to the investigation of acute lymphoblastic leukemia (ALL) has resulted in an unmasking of the heterogeneity of the disease. One of these markers, human thymus/leukemia-associated antigen (HThy-L) has been studied, isolated, purified and characterized by the applicant in collaboration with Dr. E.W. Gelfand. Recently, we succeeded in developing a competitive radioimmunoassay for quantitation of HThy/L in cell extracts and plasma.

OBJECTIVE: This study was designed to define further the specificity of HThy-L and use this marker in investigations of pathways of T-cell differentiation in normal conditions and in leukemias.

APPROACH: Using a radioimmunoassay, we have demonstrated increased quantities of this antigen in leukemic cells and plasma from all patients with T-cell ALL and a considerable number of patients with other forms of acute leukemia. Neither healthy subjects nor patients with non-leukemic disease had increased quantities of HThy-L in peripheral blood mononuclear cells. The study of established hematopoietic cell lines has demonstrated that increased quantities of HThy-L are a more stable feature of leukemic T-cells than receptors for sheep erythrocytes (E). In this application, we propose to continue our studies on the clinical application of HThy-L, the relationship of the two forms of HThy-L with the pathway and stage of differentiation of normal and leukemic cells, and the biosynthesis and cellular localization of HThy-L and its structural characterization. The investigation of the acquisition of such cell markers at various stages of differentiation and of their role in differentiation may provide the means for diagnosing and monitoring ALL and for distinguishing or selecting more specific therapy for ALL of different origins.

263. THYMIC ANTIGENS, TOLERANCE AND SELF-RECOGNITION

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Thymus grafts have been shown to produce tolerance to MHC and non-MHC antigens and to induce MHC-restricted self-recognition. It is planned to study the role of antigen presentation on thymic stroma and thymocyte populations in this phenomenon. Neonatally thymectomized mice will be given syngeneic thymus grafts, previously 'seeded' with allogeneic MHC or non-MHC antigen bearing thymocytes. The effect of these grafts on tolerance and self-recognition will be examined. The role of thymic stroma in antigen presentation will be examined in similar fashion by 'seeding' allogeneic thymic stroma or stroma bearing the non-MHC antigen with syngeneic thymocyte populations. The resulting tolerance in the above models will be examined in an effort to elucidate the mechanism(s) involved, i.e., the presence of suppressor cell populations, humoral suppressor factors, or cellular deletion mechanisms.

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264. IMMUNOCHEMICAL STUDIES ON THYMUS-LEUKEMIA ANTIGENS

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Thymus-leukemia (TL) antigens will be studied on mouse thymocytes and leukemia cells, using three immunochemical techniques that distinguish between TL molecules in the plasma membrane that: (1) exhibit TL and antigens at the surface of the cell; (2) have TL antigens that are not so exposed; (3) are within the cell. These techniques have already indicated, in our hands, that normal thymocytes may differ from leukemic cells in regard to exposure of TL antigens, and that certain TL antigens may be present in the plasma membrane of thymocytes that are not detected by cytotoxicity assays. We propose to extend these

initial observations to answer the question of whether all TL antigens of a given haplotype are expressed on the same or different molecules, to enquire further into the mechanism whereby TL negative cells acquire TL antigens as a result of eukemogenesis, and to study the process whereby TL positive cells undergo antigenic modulation in the presence of antibodies.

C. ANTIGENS ASSOCIATED WITH T AND B CELLS AND OTHER LYMPHOCYTES

265. IA-LIKE ALLOANTIGENS ON HUMAN T CELLS

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The objective of this proposal is to confirm and characterize the presence of Ia-like alloantigens on human T cells by two distinct approaches that are operational within this laboratory. The first approach utilizes HLA alloantisera and p29,34 heteroantiserum to identify Ia-like determinants which we have shown and described to exist on activated T cells. The second approach will identify T cell Ia-like alloantigens that stimulate in MLC. The determinants identified by each of these two approaches will be defined in terms of genetic characteristics, functional analysis, and disease modification. Genetic characterization will include studies to determine HLA-linkage as well as large population analysis to determine allotype specificity. Assays to determine 'Ia-like' antigen associated functions will include various in vitro cell mediated immune functions including MLC, cell mediated lympholysis (CML), primed lymphocyte typing (PLT), antibody dependent cell mediated lymphocytotoxicity (ADCC), natural killing (NK), third party suppression in MLC, lymphocyte mitogenic factor production (LMF), and macrophage dependent T lymphocyte activation. Studies to examine disease modification of newly defined antigens will involve the identification of antigens and antigen frequency with selected patient populations including testicular cancer, lymphomas and leukemias.

Preliminary experiments on T cell stimulation within a single 6-member recombinant family have identified the existence of a non-HLA MLC stimulation locus which requires HLA-B,D identity between responder and stimulator to occur (similar to Mls locus in the mouse).

We expect that amplification of our preliminary findings will lead to the characterization of new Ia-like determinants on human T cells and that the identification of such determinants may provide the proper information to allow submapping of the HLA-D region.

266. ANTIGEN EXPRESSION ON HUMAN T-LYMPHOCYTE SUBPOPULATIONS

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OBJECTIVE: To detect human T cell antigens by serological methods, to characterize and isolate them by immunochemicals means, and to correlate certain functional properties of T lymphocytes bearing these immunochemically defined antigens.

RESEARCH PLAN: To use specific xenoantisera and immunochemical techniques as probes for identifying the number and nature of T cell antigens; subpopulations of T cells would then be physically separated for structure-function correlations.

METHODOLOGY: 1) Fluorescence microscopy, 2) fluorescence activated cell sorter, 3) polyacrylamide gel electrophoresis, and 4) lymphocyte function tests (helper cell, suppressor cell, killer cell, etc).

FINDINGS, RESULTS AND CONCLUSIONS: We have 1) identified two T cell antigens with molecular weights of 16,000 and 25,000 daltons, 2) purified the 25,000 dalton antigen in milligram quantities and found it to be homologous with the Thy-1 (theta) antigen in the mouse, 3) identified differential expression of these antigens on helper and suppressor cells, 4) prepared monovalent anti-T-cell antibodies against the purified 25,000 dalton T cell antigen, 5) used the same techniques to define a new B cell antigen present on pre-B cells, mature B cells and plasma cells, 6) used these reagents for typing T cell leukemia and lymphomas in veteran patients.

CLINICAL RELEVANCE: This information has relevance in tumor immunology, transplantation immunology and in infectious diseases. Typing of surface markers in leukemia and lymphoma patients is proving useful as a means of determining prognosis and as a guide to treatment.

267. IDENTIFICATION OF DIFFERENTIATION ANTIGENS ON HUMAN LYMPHOCYTES

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We propose to prepare a series of hybrid cell lines that produce monoclonal antibodies to human lymphocytes. Our primary goal is to obtain monoclonal antibodies specific for T cells. Fusion experiments will be performed with mouse spleen cells immune to normal T cells, activated T cells, thymocytes, and cultured leukemic cell lines. Our strategy for selecting specific hybrid clones will be to utilize pairs of matched or autologus normal or activated lymphoid cells, or leukemic lines. These reagents will be used to identify functionally distinct subpopulations of T cells, especially cytotoxic T lymphocytes and their precursors, helper cells and suppressor cells. Monoclonal antibodies obtained will be used to study differentiation antigens as they are expressed by cultured hematopoietic cell lines, cultured leukemic lines and leukemic blasts. Clinically, we will use these reagents to characterize the alterations occurring in the lymphocyte subpopulations of patients with acute and chronic GVH disease. One of the central objectives will be to explore the feasibility of using monoclonal T cell antibodies for the prophylaxis or treatment of GVH disease.

268. DIFFERENTIATION OF IMMUNE SYSTEM - CELL SURFACE ANTIGENS

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The overall objectives of this project continue to be related to differentiative pathways of lymphoid cells in humans. Emphasis is on T cell differentiation and bone marrow differentiation. Differentiation is studied in both normal and malignant cell populations, e.g., in leukemia and lymphoma.

The significance of these studies relates primarily to improved understanding of the immune system and its normal function as well as the abnormal function in immunodeficiencies, autoimmune disorders, and especially in leukemias and lymphomas.

269. CELL SURFACE ANTIGENS OF LYMPHOCYTES AND TUMOR CELLS

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This study is aimed at defining and further defining new and known cell surface antigens of murine lymphocytes and spleen cells. Several approaches are used: 1. Production of alloantisera. Conventional inbred strains of mice are used and immunized with donor lymphocytes. Their sera are subsequently tested for antibody by cytotoxicity or other tests (see below). We are currently producing antisera to the products of the Thy-1, H-2, Ia, Ly-1, 2, 3, 4, 5, 6, 7, Lyb-2, 3, 4, 5, loci. In addition, several new specificities have been tentatively defined, which we have tentatively labelled Ly-9, 10, 11 (12, 13) - the earlier two not being clearly established. To define those new specificities, use has been made of the related strains 129 and LP.RIII. 2. Cell fusion for the production of antibodies. In addition to conventional means, we are now producing antisera by the cell fusion-hybridoma method, wherein immune spleen cells are fused with a myeloma, and high titred antibodies are subsequently produced in vitro. Using this method we have produced monoclonal antisera to H-2, Ia, Thy-1, Ly-1.1, Ly-2.1, Ly-9.2 and other specificities in the mouse. 3. Analysis of antisera: Serological methods. The distribution of the cell surface antigens has been analysed using not only cytotoxicity but also sensitive rosetting methods which permit the direct visualisation and isolation of cells for functional testing. In addition we have made use of the F.A.C.S. (fluorescent activated cell sorter) to analyse the distribution of all specificities - including Ly-1 and Ly-2 on T cells. 4. Functional studies. The specificities listed above are being studied in T-helper, suppressor and delayed type hypersensitivity assays and for cells responding to mitogens and in the mixed lymphocyte reaction.

BIBLIOGRAPHIC REFERENCES: Ewald, S.J., and McKenzie, I.F.C.: Immunochemical Characterisation of the Ly-5 Alloantigens on Thymocytes. Immunogenetics 8:583-587 (1979). Potter, T.A., Watt, S.M., Burgess, A.W., Metcalf, D., and McKenzie, I.F.C.: Characterisation of Surface Alloantigens of Murine Neutrophils. Immunogenetics 8:461-473 (1979).

270. ISOLATION AND CHARACTERIZATION OF MURINE LY ANTIGENS

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The proposed research is aimed at obtaining a better understanding of the role of lymphocyte alloantigens in immune recognition and 2 and 3 antigens found on murine thymocytes and a fraction of peripheral T-cells. While some progress has been made in isolating and characterizing small quantities of the Ly-2 and -3 antigens, their function remains unknown. I am interested in employing large-scale culture of murine tumor cell lines to isolate these antigens in relatively pure form so that I can characterize them biochemically and functionally.

I propose to isolate the Ly molecules by detergent extraction of purified plasa membranes and to purify them by such methods as lectin affinity chromatography and preparative gel electrophoresis. Structural analysis will include peptide mapping, N-terminal analysis, carbohydrate and amino acid composition, and orientation in intact membranes. I will examine the hypothesis that these molcules function as receptors on the lymphocyte surface by reconstituting them in liposomes and assaying for inhibition of cytotoxicity and for specific binding to cells of known cell-surface phenotype.

These studies are of interest to the problem of cell-cell interaction in general, as well as to the question of what the in vitro and in vivo requirements are for tumor cell recognition and lysis. They are also potentially relevant to the study of the native killer (NK) cells, a population of cells believed to be important in immune surveillance, whose cell-surface phenotype is not yet characterized.

271. SEROLOGICAL DEFINITION OF T CELL IA ANTIGENS

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The I region of the H-2 complex is known to control a number of immune phenomena, some of which are thought to be strictly T cell functions, such as the MLR, QVHR, and interaction with macrophages, and some of which involve B cells, such as T-B collaboration and antibody responses to defined antigens. Although I region products (Ia molecules) are easily found on B cells, early studies indicated that T cells do bear Ia molecules, and at least one of these antigens is different from those found on B cells. The objective of this project is to study Ia antigens on certain T cell subpopulations by serological and biochemical techniques, some of which will be done as a collaborative effort. For this purpose, the enrichment of three T cell subpopulations will be obtained: an I-J(plus) suppressor cell, an I-J(plus) helper cell, and a presumed I-C(plus) MLR suppressor cell. The enrichment methods for the first two are already published, and it is hoped that similarly simple methods will also yield the third. These subpopulations will be used in the following ways: (1) existing antisera will be used for serological characterization; (2) a collaborating lab will analyze the cell-surface Ia biochemically; (3) antisera will be produced in congenic mice and tested as in number 1; (4) the known functions of the subpopulations will be tested to evaluate the effects of the above antisera.

272. T CELL SUBSETS AND MAREK'S DISEASE VIRAL ONCOGENESIS

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Two highly inbred MHC-identical lines of chickens differ markedly in susceptibility to viral oncogenesis, and express contrasting T cell surface antigens determined by the independent Ly-4 and Th-1 loci. These antigens may be markers for partially overlapping subsets of T cells, and there is evidence that one of these loci, and perhaps both, are associated with resistance to Marek's disease oncogenesis. The extent to which these surface antigens are markers for distinct subsets of central and peripheral T lymphocytes will be analyzed during embryogenesis and in growing chickens, using immunofluorescent techniques and double labeling.

In vitro methods will be used to investigate whether the expression of suppressor and cytotoxic activity of immunocompetent cells is associated with the expression of inherited resistance to viral oncogenesis in Marek's disease, and attempts will be made to relate these functional properties to the T cell subsets.

273. IMMUNOGENETIC MAPPING OF THE CELL SURFACE

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The summary for this project is under entry no. 259.

274. LYMPHOCYTE INTERACTIONS, RECEPTORS AND FUNCTIONS

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A B cell leukemia (L2C) of inbred strain 2 guinea pigs and a new myelogenous leukemia (G-13) of inbred strain 13 guinea pigs are being investigated as models of human leukemia. The chemical nature of the tumor specific transplantation antigen (TSTA) of the L2C leukemia is being investigated by immunization protection tests in syngeneic animals. The TSTA of the L2C leukemia is unusual in that it is of low molecular weight and is extremely resistant to heating. The G-13 leukemia is being examined for the presence of a TSTA and cell surface markers. As in the human, the myeloblasts of this leukemia are Ia positive. The immune functions of these cells are also being investigated.

Patients with systemic lupus erythematosus (SLE) have a defect in the development of Con A induced suppressor cells. Moreover, the sera from lupus patients (more particularly the IgM fraction) can induce suppressor cell defects in populations of normal lymphocytes. T gamma and T non-gamma cells mediate different types of suppression; certain lupus sera are specifically cytotoxic for T gamma cells. Finally T cells able to proliferate in response to autologous non-T cells act as precursors of Con A induced suppressor cells.

BIBLIOGRAPHIC REFERENCES: Gelfand, M.C., Frank, M.M., Green, I., and Shin, M.L.: Binding sites for immune complexes containing IgG in the renal interstitium of man and other species. Clinical Immunology and Immunopathology 13: 19, 1979. Nilsson, S.F., Edelson, R., Mann, D., Green, I., and Waxdal, M.J.: Concanavalin A binding proteins on the surface of human malignant and normal lymphocytes. Scand. J. Immunol. In press, 1979.

275. FINE SPECIFICITY MARKERS OF HUMAN T CELL ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

276. A STUDY TO DEFINE A NEW LEUCOCYTE HISTOCOMPATIBILITY SYSTEM IN MAN

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OBJECTIVE: To define B lymphocyte alloantigens in man and to establish their relevance to lymphoproliferative disorders.

APPROACH: Sera from ante-natal, multitransfused, transplanted and leukemic patients are screened against cells from patients with CLL and other B cell proliferative diseases, and their patterns of reactivity analyzed with reference both to the newly defined DR antigens and also to the particular diseased cells being used in the hopes that some sera may prove useful diagnostically.

PROGRESS: This work has produced many antibodies which can be used for typing B cell antigens and some of these were used in the 7th Histocompatibility Testing Workshops, Oxford 1977.

277. ANALYSIS OF SURFACE STRUCTURES OF HUMAN AND MURINE B LYMPHOCYTES, PLASMA CELLS AND LYMPHOMA CELLS

Hartmann, K., Moldenhauer, G., Philipps Universitat Marburg, Inst. for Experimental Immunology, *Deutschhausstr. 1, D3550 Marburg an der Lahn, Federal Republic of Germany*

OBJECTIVE: We are studying the regulation of B lymphocytes, especially the interactions between macrophages and B lymphocytes, and the participation and role of specific proteinases (partly derived from the complement system) during the induction phase.

APPROACH: In the project described here we want to detect and to characterize various surface molecules (other than Ig molecules and MHC-coded antigens) of human and murine B lymphocytes, and their quantitative and qualitative changes during differentiation. We have selected monoclonal antibodies directed against B lymphocyte- and/or macrophage-specific antigens; we shall analyze their distribution and quantitative patterns, and the formation of neoantigens or antigen complexes during stimulation, and we shall characterize these structures with biochemical methods (labelling, affinity chromatography, two-dimensional PAGE, etc.) and determine their influence in functional assays in vitro and in vivo. The analysis with monoclonal antibodies (in collaboration with other groups in Europe, U.S.A. and Australia) will help us to learn more about the regulatory events on the cell surface of B lymphocytes; it will be extended to include clinical analysis (and probably a study of the influence) of lymphoma cells.

PROGRESS: We have already selected 7 hybridomas (rat plasma and mouse myeloma cells) which recognize mouse B cell (but not T cell) structures other than Ig or H2. We are now characterizing the recognized antigens biochemically, and we are just starting to select (by ELISA and fluorescent techniques) monoclonal antibodies directed against human B lymphocytes and plasma cells. Mice were immunized with B lymphocytes, plasma cells resp. Raji lymphoma cells.

278. CHARACTERIZATION OF HUMAN B LYMPHOCYTE DIFFERENTIATION ANTIGENS

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The research project is aimed at more precise definition of surface membrane markers for B lymphocyte differentiation in the human. A second goal is the identification of membrane components involved in activation of B-cells for antibody synthesis. Finally, a model for the development of B lymphocyte neoplasia will be tested with the markers developed in the initial phase of the study.

The methods involved in the proposal are the preparation and testing of conventional and monoclonal antibodies based on strategies developed in our parallel work on the mouse and the concept that there will be a high degree of molecular homology between murine and human differentiation antigens. Binding studies and correlation of new markers with known B-cell surface receptors will be done using two-color analysis on the dual laser fluorescence activated cell sorter. Membrane components will be characterized using two-dimensional gel electrophoresis. Normal human B-cells, as well as lymphocytes from chronic lymphocytic leukemia and lymphoma patients, will be analyzed for the sequence of appearance of B-cell markers and their presence or absence during pre-neoplastic states. Assuming we identify B-cell receptors for growth-regulatory factors, a hypothesis which states that an initial mutation leads to a loss of regulation of cell proliferation because of the absence of a membrane receptor class can be tested. The role of antigen in the unregulated growth process also will be tested with anti-idiotypic antibodies to characterize clonal expansion.

279. STUDIES OF LYMPHOCYTE-DEFINED HLA AND B-CELL ANTIGENS

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Human somatic cell mutations arising in vivo in peripheral blood lymphocytes (PBLs) are quantitatively detected by a test which enumerates variant purine analogue (8 azaguanine, 8 AG; 6 thioguanine, 6 TG) resistant (AGr; TGr) Lesch-Nyhan (LN)-like PBLs in non-LN individuals. AGr and TGr PBLs are recognized in vitro by their resistance to analogue inhibition of phytohemagglutinin (PHA) induced 3HTdr incorporation. Variants are enumerated autoradiographically.

Recent studies have suggested that some apparently TGr PBL's detected autoradiographically are phenocopies, i.e., non-mutants. Increasing intervals in vivo in selective agent, changing the viability marker to 3HUdr (RNA synthesis) and insuring that PBLs are at the G0 cell cycle phase at initiation of in vitro assay should eliminate phenocopies.

Human HLA loss variant PBLs arising in vivo will be studied in an analogous fashion. Quantitative measures for their enumeration will allow this marker system also to be used for direct human mutagenesis studies. HLA loss variant PBLs, and continuously growing T-cells established from them, may be used as reagents in HLA testing.

Human T-PBLs are propagated in vitro using T-cell growth factor (TCGF) in both suspension and clonal soft agar cultures. TGr and HLA loss variant PBLs arising in vivo will be propagated in vitro with TCGF, the stability of the variant phenotypes will be assessed, and appropriate characterizations to document the mutant nature of the variants will be performed. Continuously growing T-PBLs maintained with TCGF will provide targets for an in vitro mutagenesis system to parallel the in vivo systems.

Rat PBL systems analogous to those described for humans are being developed.

280. STUDY OF TRANSPLANTATION AND TUMOR ANTIGENS

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We have continued our research leading to identification and characterization of the antigenic factors making up the major histocompatibility locus in man that are important in organ transplantation. We have identified a number of additional B cell specific antigens of the HLA-Dr system and have studied their inheritance in families. We have completed studies on a number of black Americans and have shown that 5 new B cell specificities are identified in this patient population. In contradistinction to Caucasians, black Americans typed for HLA-D with homozygous typing cells failed to exhibit strong linkage disequilibrium between D and Dr types. This could prove to be important in transplantation between these ethnic groups. We have additionally continued our studies concerning the association of tumor associated antigens present on the cell membrane and have now prepared monospecific antibodies to human melanoma antigen and have characterized a human anti-human alloantibody, a chimp anti-human xenoantibody, and a monoclonal antibody prepared by fusion of mouse myeloma cells with specifically sensitized human lymphocytes against melanoma. These antisera specifically localize human tumors in the in vivo setting. We have begun developing methodology for in vitro induction and assay of blastogenesis, cytotoxic lymphocytes, and suppressor cell generation using appropriate tumor cells or PBL from HLA non-identical individuals as targets. Using our mono-reactive anti-tumor antibodies we have completed immunofluorescence and immunoprecipitation studies providing physical-chemical analysis of the antigens present on the surface of human melanoma tumor cells. Cocapping and coprecipitation studies using these reagents are in progress to assess the physical-chemical association of the tumor antigens to the Dr antigens and MHC antigens expressed on normal cells.

BIBLIOGRAPHIC REFERENCES: Avent, J., Vervaert, C., and Seigler, H.F.: Non-Specific and Specific Active Immunotherapy in a B16 Murine Melanoma System. J. Surg. Onc. 12: 87-96, 1979. Seigler, H.F., Cox, E., Mutzner, F., Shepherd, L., Nicholson, E., Shingleton, W.W.: Specific Active Immunotherapy for Melanoma. Ann. Surg. 190: 366-372, 1979.

281. DIFFERENTIATION AND VIRAL ANTIGENS ON PRIMATE B LYMPHOID CELL LINES

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The summary for this project is under entry no. 144.

282. IMMUNE RESPONSE TO SYNGENEIC LEUKEMIC B CELL ANTIGENS

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I seek to identify the tumor-associated transplantation antigen(s) (TATAs) present on the transplantable B lymphocyte leukemia (L2C) of strain 2 guinea pigs. The initial step will be to define the specificity of the L2C antibodies and the L2C cytotoxic T lymphocytes that I have detected in the sera and spleen of syngeneic animals immunized with L2C tumor cells. To achieve this, the in vitro lysis of L2C tumor cells with L2C antibodies or cytotoxic T cells will be inhibited using F(ab')2 antibodies specific for either the L2C surface IgM idiotype (sIgM id), the L2C B antigen or the L2C Ia antigen. Moreover, the isotype of the L2C antibodies, and the presence of Ia antigens or Fc receptors on L2C cytotoxic T lymphocytes, will be determined. These studies will enhance our unnerstanding of mechanisms of the immune response to tumor antigens.

To analyze more rigorously whether the sIgM id on L2C cells can function as a TATA, normal syngeneic animals will be id-suppressed during their immunological maturation by injection with anti-id antibodies. These animals will be studied for their capacity to mount an anti-id response against the L2C leukemia. Lymphoid cells from animals resistant to the L2C leukemia will be transferred to syngeneic normal adult or tumor bearing animals to determine the capabilities of the anti-id clones to prevent the dissemination of the L2C leukemia.

Since most experimental tumors do not have sublines which differ significantly in their immunogenic potency, the L2C leukemia provides a unique opportunity to determine how a subtle molecular difference on the plasma membrane of tumor cells can modulate their immunogenicity. The membranes of Ia-positive and Ia-negative L2C tumor cells will be disrupted, and the various fragments will be examined for their immunogenic potential. The membrane fragments will also be analyzed chemically and serologically for the presence of sIgM id, B antigen, or Ia antigens. In addition, the structure of the sIgM on both L2C sublines will be investigated along with the association properties of Ia antigens.

283. MEMBRANE ANTIGENS OF NORMAL AND MALIGNANT HUMAN LYMPHOCYTES

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OBJECTIVE: Identification of antigens specific to normal, activated and malignant T and B lymphocytes, in order to define functional subsets of these cells.

APPROACH: Production of monospecific antibody to different membrane antigens by the hybridoma technique using as immunogen purified human T and B cells.

PROGRESS: Nine monoclonal antibody recognizing antigens present in various proportions in T and B lymphocytes have been obtained and are currently being characterized.

284. IDENTIFICATION OF HUMAN LYMPHOCYTE SURFACE ANTIGENS

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The objective of this proposal is to prepare monospecific antisera against human lymphoid cell surface antigens. Antigens specific for normal human thymus-dependent (T) and thymus-independent (B) lymphocytes and tumor-specific lymphoid antigens reactive with xenoantisera will be identified by biochemical and serological techniques. Cell hybrids between human T and B cell lines and a mouse lymphoma cell line will be prepared. Clones of cell hybrids, which have lost all but one of their human chromosomes and express only the human surface antigen of interest, will be selected by cloning out spontaneous variants or by cytotoxic immunoselection. These cells will then be used to immunize mice to give monospecific antisera against the human antigen. Since lymphoid antigens show a high degree of species specificity, attempts will be made to raise large quantities of effectively monospecific antisera against the human cell surface component by immunization of larger animals with the hybrid cells. The significance of this work lies in the new approach used to study human lymphocyte antigens in which biochemical, serological and somatic genetic techniques will be used in combination to achieve a depth of analysis not presently attained. The antisera produced will allow further detailed studies of normal and malignant human lymphocyte physiology to be carried out and may be useful as diagnostic or therapeutic reagents in human lymphoid diseases.

285. CELL SURFACE ANTIGENS IN NEOPLASTIC AND AUTOIMMUNE DISEASE

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In collaboration with Dr. Ronald Levy at Stanford, monoclonal antibodies were produced that could discriminate between a T-cell leukemia (T-ALL) and a B-lymphocyte cell line from the same patient (Levy, 1979). Approximately 60 hybridoma clones were isolated which produced monoclonal antibodies that could be divided into the following categories: (1) antibody that binds to T-ALL but not to B cell lines or peripheral blood lymphocytes (PBL); (2) antibody that binds to both T-ALL and PBL but not to B cells; (3) antibodies that bind to both T and B cells but immunoprecipitate different size molecules from each cell. For each class of antibodies, using immunoprecipitation and gel electrophoresis we have obtained preliminary data including antibody binding profiles, fluorescent staining patterns and characterization of the antigens recognized. We have noted that a monoclonal antibody may immunoprecipitate different molecular weight antigens from different cell types (and in disease states). To extend these results, peptide maps of immunoprecipitated antigens will be performed. We will extend our library of monoclonal antibodies to obtain additional antibodies that react with subpopulations of lymphocytes.

These monoclonal antibodies will be used to classify lymphocytes into subsets based on cell surface antigens in both normal and disease states. These reagents may prove useful not only in classifying the types of lymphocytes involved in inflammatory reactions and lymphoproliferative diseases, but also in early detection of disease relapses. An additional advantage of monoclonal antibodies is that high titers and large amounts of antibody can be produced that make it suitable for widespread use as a detecting reagent. Finally, by detecting specific antigens associated with disease states, monoclonoal antibodies can potentially play a major role in elucidating the pathogenesis of autoimmune diseases.

286. THE ISOLATION AND CHARACTERIZATION OF TISSUE AND TUMOR ANTIGENS IN DOGS

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OBJECTIVE: The project is related to a study, carried out in our laboratory, on the immunotherapy of malignant tumors in dogs.

Tumor material was collected in cooperation with veterinarians and tumor cell lines were established in vitro. The tumor bearer lymphocytes were typed for lymphocyte defined and serum defined histocompatibility antigens, and attempts were made to type tumor cells as well.

A biochemical approach to the investigation of tumor cell surface antigens is necessary to solve the problem of the specificity of tumor associated antigens (TAA) and to see if there is any relationship between TAA and histocompatibility antigens (DLA) on the surface of dog tumor cells.

APPROACH: DLA antigens on the surface of normal tumor cells are characterized by immunoprecipitation with DLA typing sera. 125I labeled membrane antigen preparations are used in case of normal lymphocytes, whereas cell lines are also labeled with 35S methionine. The precipitates are analyzed by slab gel electrophoresis and fluorography.

Patterns obtained with membrane antigens of tumor cells and the corresponding tumor bearer lymphocytes are compared. In this way quantitative and qualitative changes in DLA antigens on tumor cells may be detected.

PROGRESS: A careful estimation of the molecular weight of DLA antigens obtained in immunoprecipitation experiments with detergent solubilized material can be made; DLA consists of a heavy chain of 44,000 and a light chain of 12,000 daltons.

Differences were found in the gel patterns of a melanoma tumor cell line and the corresponding lymphocytes. The techniques are not sensitive and reproducible enough for any firm conclusions to be drawn based on these data.

Sera will be purified by absorption and an anti-dog B2 microglobulin serum will be produced as a positive reference serum in the immunoprecipitation experiments.

287. GENETICS AND BIOCHEMISTRY OF MOUSE ALLOANTIGENS

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I plan to investigate a segment of mouse chromosome 17 situated near the major histocompatibility complex (MHC). The objectives are to elucidate the organization of genetic information; define the extent of polymorphism and/or number of loci encoding cell surface antigens in this region; determine the expression of these antigens on various populations of lymphocytes in order to identify markers of functional subsets of lymphocytes; then investigate the biochemical nature of these antigens to determine their evolutionary relationship. In order to accomplish this, I will initiate experiments to search for genetic recombination in this region. These and other recombinants will be serologically analyzed by cross-immunization and absorption of antisera: The biochemical characteristics will be established by immunoprecipitation of antigen and analysis by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), peptide mapping and partial N-terminal amino acid sequence analysis.

288. FUNCTIONAL SURFACE ANTIGENS OF T- AND B-CELL TUMORS (MICE, RABBITS)

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This is part of a broader project. A summary of this subproject is not available.

289. CELL SURFACE ANTIGENS ON MALIGNANT LYMPHOCYTES

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Possible antigen interactions occurring between the tumor-associated surface antigen (TASA) and the K and D regions of the major histocompatibility complex (H-2Kk,Dk) on the surface of malignant line Ib lymphocytes are currently being evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoelectron microscopy (IEM). Indirect immunoprecipitation of solubilized, radiolabeled membrane antigens of Ib lymphocytes employing both rat anti-H-2Kk,Dk and rabbit anti-Ib TASA and their respective antisera has demonstrated that both proteins share a 16,000 dalton moiety, thus suggesting the 'altered-self' model at the target cell level. These observations are currently being confirmed by direct observation at the ultrastructural level employing a double-labelling technique. Studies concerning the distribution and valence of the Ib TASA indicate that the antigen is randomly distributed on all malignant lymphocytes and occupies about 1% of the total surface area. Similar analyses of the distribution and valence of the H-2Kk,Dk antigens by IEM have been initiated but are too preliminary for comment.

D. LYMPHOMA, SARCOMA, AND MYELOMA-ASSOCIATED ANTIGENS

290. HUMAN LEUKEMIA- AND LYMPHOMA-ASSOCIATED ANTIGENS

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The summary for this project is under entry no. 222.

291. DETECTING PAUL-BUNNELL ANTIGEN IN LYMPHOMA AND LEUKEMIA

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The summary for this project is under entry no. 217.

292. IDENTIFICATON OF NEOPLASTIC ANTIGENS AND IMMUNOLOGICAL FOLLOW-UP OF PATIENTS WITH LYMPHOMAS, LEUKEMIAS AND SOLID TUMORS

Del Giacco, G. S., Mantovani, G., Locci, F., Cengiarotti, L., Leone, A. L., Loy, M., Mura, E., Pau, L., Universita degli Studi di Cagliari, Chair of Clinical Immunology, *Cagliari, Italy*

The summary for this project is under entry no. 214.

293. HUMAN LEUKEMIA AND LYMPHOMA - LYMPHOCYTE MEMBRANE ALLOANTIGENS AND MEMBRANE MARKERS (ABBREV)

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The summary for this project is under entry no. 211.

294. IMMUNODIAGNOSIS OF ASYMPTOMATIC MALIGNANT LYMPHOMAS

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OBJECTIVE: The early detection of malignant lymphomas in an asymptomatic state.

APPROACH: The presence of a circulating lymphoma associated antigen (LAA) in lymphoma patients has already been reported. A double-blind trial showed 98% correlation indicating that the immunological test using LAA-anti LAA system may prove specific for lymphomas. The antigen has also been detected in the body fluids (via) saliva, CSF and urine of lymphoma patients.

The cytotoxicity of the LAA antiserum was tested by trypan blue exclusion method using the peripheral lymphocytes of normals and of patients with various types of cancers, including lymphomas. Immunofluorescence study was carried out using malignant lymphoid tissue of lymphoma patients and controls.

The molecular weight of LAA has been found to be in the region of 140,000 by gel filtration employing reference proteins like BSA pepsin and ferritin.

PROGRESS: The trypan blue exclusion method and the fluorescein isothiocyanate technique revealed that the LAA antiserum is cytotoxi. to the peripheral lymphocytes and lymphoid cells of lymphoma patients.

A radioimmunoassay procedure is being developed for the quantitative estimation of circulating LAA in normals and in patients with various types of cancers and lymphomas.

295. MALIGNANT BEHAVIOR AND CELLULAR ANTIGEN EXPRESSION

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The summary for this project is under entry no. 233.

296. TUMOR-ASSOCIATED ANTIGENS AND NEOANTIGENS ON HUMAN MALIGNANT LYMPHOID CELLS AND LYMPHOBLASTOID CELL LINES

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This project is aimed at the study of tumor-associated cell surface antigens of human lymphoid malignancies and neoantigen expression of cultured lymphoblastoid cell lines. For this purpose, xenogeneic monospecific antibodies will be generated in vitro using the mouse spleen fragment culture system, as developed by Klinman. Fragments will be isolated which produce homogeneous, monoclonal antibodies recognizing specifically human malignant lymphoid cells or cultured lymphoblastoid cells, but not normal lymphoid cells. The objective will be to determine the number of tumor-associated antigens and neoantigens on malignant lymphoid cells and cultured lymphoblastoid cells, respectively, and the degree to which these antigens are shared by related tumor cells and cell lines.

In addition, because antibody producing fragments have a limited life span in vitro, we will attempt to expand the specific antibody producing clones in such fragments by fusing them to mouse myeloma tissue culture cell lines, as described by Kohler and Milstein. In such a way, hybrid cell lines can be grown to produce monospecific anti-tumor antibody. These antibody producing hybrid cell lines can be propagated indefinitely and lead to the large scale production of specific antibody. Such monospecific anti-tumor antibodies produced in vitro may be applicable to immunodiagnosis and immunotherapy.

297. PRODUCTION OF MONOSPECIFIC LYMPHOMA ANTIBODIES

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The summary for this project is under entry no. 247.

298. ANTITUMOR ANTIBODIES GENERATED IN VITRO

Levy, R., Finn, O. J., Dilley, J., Zipf, T., Stanford University, School of Medicine, Dept. of Medicine, *Stanford, California*, 94305, United States of America

In this project we will be attempting to study cell surface antigens which are specific to tumors. For this purpose we have adapted a technique for the production of antibodies of restricted heterogeneity in vitro. The objectives here will be to define the antigenic relationships between different closely related murine lymphoid tumors, and to determine the degree of overlapping in the array of tumor specific antigens of these different tumors. In addition, we will be attempting to produce antibodies which recognize human tumor specific antigens, particularly in leukemia and lymphoma. These antibodies against human tumor antigens may find application in immunodiagnosis and immunotherapy.

299. LYMPHOMA IMMUNOGLOBULINS AS TUMOR SPECIFIC ANTIGENS

Levy, R., Stanford University, School of Medicine, Dept. of Medicine, *Stanford, California*, 94305, United States of America

In this project, we will be attempting to study cell surface antigens which are specific for tumors. For this purpose, we have adapted a technique for the production of antibodies of restricted heterogeneity in vitro. The objectives here will be to define the antigenic relationships between different closely related lymphoid tumors and to determine the degree of overlapping in the array of tumor specific antigens of these different tumors. In addition, we will be attempting to produce antibodies which recognize human tumor specific antigens, particularly in leukemia and lymphoma. These antibodies against human tumor antigens may find application in immunodiagnosis and immunotherapy.

300. EXTRACTION, SOLUBILIZATION AND CHARACTERIZATION OF MEMBRANE ANTIGENS OF MURINE LYMPHOMAS

Pierotti, M. A., Torre, G. D., Miotti, S., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To identify on cell membranes the target structures for the natural immune response against murine lymphosarcomas and to study their correlations with viral and histocompatibility antigens.

APPROACH: The extraction has been carried out by passing the tumor tissue through a mechanical press, and by recovering the cell membrane from the homogenate by differential centrifugation. The solubilization of the particulate extract was carried out by Na DOC. The characterization of the extracted antigens will be made with normal sera of C3Hf mice which have been found to possess a natural response against lymphoma membrane antigens and endogenous type C virus antigens. Alloantisera and xenogeneic antisera specific for leukemia virus proteins will also be used. The relationship between the various antigenic structures of the leukemia cell membrane will be evaluated by immunochemical and immunoelectronmicroscopic studies, as well as by biological tests.

301. CHARACTERIZATION OF ANTIGEN ON SPONTANEOUS LYMPHOMA CELL MEMBRANES

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Tumor antigens of the transplantation type have been identified on the membranes of malignant cells of spontaneous lymphomas occurring in Syrian golden hamsters. This disease is horizontally transmitted to susceptible animals by unidentified but highly infectious agent with an incidence greater than 50 percent. This lymphoreticular malignancy in random bred and inbred hamsters constitutes a unique model for the investigation of tumor membrane-associated antigens and their role in the ability of a spontaneous neoplasm to escape immune elimination. The research described in this proposal is directed toward the purification and immunochemical characterization of the lymphoma membrane-associated antigen(s). Solubilization of the antigen(s) will be attempted with nonionic detergents, limited papain digestion, or extraction with M KCl. Subsequent fractionation of membrane extracts will involve gel filtration and ion exchange chromatography in combination with affinity, lectin affinity or hydrophobic chromatography. Specific in vitro and in vivo assays compatible with these methods will be developed for monitoring the antigenicity and transplantation rejection activity during purification. The origin of the lymphoma antigen will be determined as either 1) an antigen of the causative agent, 2) an agent-directed membrane antigen, 3) a non-agent directed tumor-specific transplantation antigen, or 4) an antigen of fetal origin. True tumor-specific transplantation rejection activity will also be used in preliminary studies defining the humoral and cellular immune responses elicited against the lymphomas. The ultimate objective is a better understanding of the role of tumor-specific membrane antigens in the success of neoplastic disease.

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302. PURIFICATION OF HUMAN SARCOMA HETEROPHILE ANTIGENS

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This laboratory has identified 3 sarcoma-associated antigens, S1, S2 and S3, which are present in human sarcoma cells growing in tissue culture. In addition, the classical heterophile substances, Forssman and serum sickness antigens, have been shown to be associated with sarcoma and other forms of malignancy. The titers of antibody to Forssman antigen are significantly elevated in sarcoma patients and the prevalence of antibody to serum sickness antigen can be observed. Recent immunologic studies have shown that both S1 and S3 are present also in boiled guinea pig tissue and, therefore, 'heterophile' in nature. They are not present in sheep red blood cells, whereas the classic heterophile antigen, Forssman, infectious mononucleosis and serum sickness antigens are.

Efforts to isolate human tumor antigens have, in general, been hindered by a lack of sufficient substrate for biochemical purification. The presence of antigen capable of absorbing antibody to S1 and S3 in guinea pig kidney tissue presents an unusual opportunity for the biochemical isolation of substances sharing reactivity with human tumor antigens without concern for the quantity of tissue needed. Once purified antigens are available, radioimmunoassays can be prepared which can be used to define more precisely the distribution of antigens in human tissues and the prognostic implications of changes in

antigen and antibody levels. Since multiple antigens are present in guinea pig kidney to which patients may have antibody, this study design provides for first cataloguing the distribution of such antigens in fractions prepared by extraction procedures. The antigens found will then be characterized to determine whether they represent known substances or, if new, whether there is any evidence of tumor-relatedness. Purification of S1, S3 and other relevant antigens will then be undertaken. It is anticipated that this study will provide important new insights into the nature of antigens which frequently provoke cross-reactive but yet clearly tumor-related immune responses in patients with sarcoma and other solid tumors.

303. STUDY OF THE BIOLOGICAL ACTIVITY OF SOLUBILIZED AND PURIFIED MEMBRANE EXTRACTS

Colnaghi, M. I., Miotti, S., Pierotti, M. A., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To evaluate, in vivo and in vitro, the biological activity of tumor associated membrane antigens extracted from murine lymphosarcomas.

APPROACH: Membrane preparations are obtained by passing lymphosarcoma tissue through a mechanical press and by differential centrifugation of the obtained homogenate. The preparations will be used as a particulate or in a soluble form to immunize (according to various schedules) syngeneic mice whose immune response will be evaluated as to in vivo tumor growth resistance and as to in vitro humoral and cellular antitumor cytotoxic reactivity. The same membrane preparations will be also tested for the capacity of inducing secondary in vitro sensitization of spleen cells from mice previously treated with tumor cells or with the same extracts.

PROGRESS: The secondary in vitro sensitization has been achieved by using membrane extracts ina histoincompatible system.

304. CELL SURFACE ANTIGENS OF MOUSE SARCOMAS

DeLeo, A. B., Old, L. J., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

Little is known of the biochemical nature or genetic origins of the individually distinct transplantation antigens which characterize chemically induced sarcomas of inbred mice. Serological reagents detecting these antigens would clearly facilitate their analysis. We have successfully defined two serologically distinct cell surface antigens of methylcholanthrene-induced sarcomas of BALB/c origin, Meth A and CMS4. The exceedingly limited tumor distribution of these antigens resembles in this respect the individually distinct transplantation antigens of these tumors.

We now plan to apply hybridoma methodology to this analysis. Monoclonal antibody will be screened for reactivity to cell surface antigens using several serological assays and a panel of normal and transformed cell types. The initial aim of our analysis will be to classify the range of cell surface antigens being identified. Our second aim will be to obtain a molecular characterization of transformation-related and individually distinct antigens. This will involve one- and two-dimensional SDS-PAGE and peptide analysis of proteins immunoprecipitated by monoclonal antibodies from surface and metabolically labeled target cells.

Once a family of individually distinct antigens has been defined and characterized, it will then be possible to explore their genetic origins and relationship to the distinct transplantation antigens of chemically induced sarcomas.

305. MIXED LEUKOCYTE TUMOR REACTION IN SYNGENEIC SYSTEMS

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We plan to characterize the molecular nature of the tumor associated antigens involved in the stimulation of syngeneic lymphocytes. Both 3H-thymidine incorporation and cell mediated cytotoxicity will be measured. The tumor system that will be used is the spontaneous reticulum cell sarcoma of SJL/J mice. In addition to the bioassays used, we plan to isolate antigens by co-precipitation with nonspecific antisera and separation on SDS gel electrophoresis. Furthermore, in some instances peptide mapping of isolated molecules will be undertaken. The relationship between antigens isolated from normal and neoplastic cells will be established.

306. INAPPROPRIATE ANTIGENS ON SJL/J RETICULUM CELL SARCOMA

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The main objective of this project is to characterize the immunological properties and molecular nature of inappropriate antigens found on reticulum cell sarcoma derived from SJL/J mice. The role of these antigens in tumorogenesis is also being investigated. The presence of alien H-2 antigens was determined by several immunological tests, including cell-mediated cytotoxicity, complement-dependent cytotoxicity, and immunofluorescence, and by opsonization. The biochemical nature of these antigens was determined by 2D gel analysis.

307. IDIOTYPIC MARKERS ON HUMAN MYELOMA PROTEINS

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The aim of the project is to determine if individually specific idiotypic markers on human myeloma proteins can be employed as tumor specific antigens in patients with monoclonal gammopathy.

Correlations will be sought in each patient between diagnosis, clinical progress and prognosis, and studies of residual bone marrow lymphoid cells and serum myeloma protein, identified by the idiotypic markers specific for the myeloma protein of that patient. Serial studies of such patients with these methods should permit earlier recognition of impending relapse, presumably indicated by increased numbers of idiotype-bearing cells and increases in serum levels of the myeloma protein.

This may permit restoration of therapy or a change of drugs, based on the presumption that the therapeutic response will be improved if the patient is treated at the stage of a smaller tumor mass.

308. ANTIGENIC ANALYSIS OF HUMAN SOLID TUMORS WITH MONOCLONAL XENOANTIBODIES

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OBJECTIVE: To raise monoclonal antibodies against cell surface antigens of human carcinomas and characterize antigens in order to compare their expression on normal and malignant cells.

APPROACH: Rats or mice are immunized with human colon carcinoma cells from tissue culture or primary tumor membranes. Immune spleen cells are fused with rat or mouse myeloma cells and the hybrid cells are tested for the production of antibodies to immunizing tumors. Positive hybrid cells are cloned and the monoclonal antibodies are tested for their specificities by a panel of human tissue culture cell lines, tissues and sera from patients. Antibodies that react with tumors but do not react strongly with normal tissues are selected and the antigens are characterized for their expression on tumor and normal cells as well as for their biochemical natures.

309. STUDY OF HUMAN MULTIPLE MYELOMA CELL ANTIGENS

MacKenzie, M. R., University of California, Davis Campus, School of Medicine, Dept. of Medicine, *Davis, California,* 95616, United States of America

Plasma cell tumors from man and mouse will be established in tissue culture. These cells will be used as source material for the isolation and characterization of plasma cell antigens. Antigens will be isolated and characterized by means of cell surface labeling followed by reaction with anti-B, T or plasma cell antisera, and the products analyzed by polyacrylamide gel electrophoresis. Antigens will be isolated and characterized by means of potassium chloride extraction gel chromatography, ion exchange chromatography and polyacrylamide gel electrophoresis. The immune response to tumor antigens by peripheral blood

leukocytes will be characterized as to the nature of the responding cell and magnitude of the immune response by use of cell separation techniques. Suppressor cell populations for the immune response to plasma cell antigens, immunoglobulin production and mixed lymphocyte reactions will be isolated, their activity quantified and the mechanism of action explored. These studies will involve cell separation techniques, the use of cell surface markers, including heterologous antisera to B, T and plasma cells, cell surface labeling and immunochemical characterization.

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310. PATHOBIOLOGY OF MYELOMA AND ANTI-IDIOTYPIC IMMUNITY

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Surface immunoglobulins on malignant B cells have idiotypic determinants. In addition to viral antigens, these determinants function as tumor-specific antigens and therefore as target sites for the immune system. We propose to analyze the factors which are essential for tumor-specific humoral antibodies and T cells to suppress myeloma growth in vitro and to determine the heritable and nonheritable phenotypic changes which make tumor cells susceptible or resistant to such immunity. Anti-idiotypic immunity is mediated by different kinds of antibodies and T lymphocytes. We plan to investigate the effects of this immunity on the in vitro growth of the S107 myeloma. We have several culture sublines available which differ in amount of surface immunoglobulin and immunoglobulin secretion. We will use a cloning system to measure effects on cloning efficiency, clone size and rate of growth, and to detect changes in the quantity or the idiotypic specificity of the target antigen secreted. Different classes of antibody will be tested for their ability to stimulate or inhibit tumor cell growth. Similarly, the effects of subpopulations of T cells will also be investigated. Clones that have escaped idiotype-specific immune destruction will be isolated. The frequency of escape variants showing heritably increased resistance to anti-idiotypic immunity will be determined. The mechanisms for the increased resistance will be analyzed. The study of this experimental model is expected to provide useful information for developing specific immunotherapeutic procedures.

BIBLIOGRAPHIC REFERENCES: Leibson, P.J., Panem, S. and Schreiber, H. Clonal evolution of myeloma cells leading to quantitative change in immunoglobulin secretion. Proc. Am. Assoc. Cancer Res. 20: 97, 1979. Leibson, P.J., Loken, M.R., Panem, S. and Schreiber, H. Clonal evolution of myeloma cells leads to quantitative changes in immunoglobulin secretion and surface antigen expression. Proc. Natl. Acad. Sci. USA 76: 2937-2941, 1979.

E. MELANOMA- AND SKIN-ASSOCIATED ANTIGENS

311. CHARACTERIZATION OF HUMAN MELANOMA-ASSOCIATED ANTIGENS

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OBJECTIVES: 1) To gain a more detailed characterization of the antigenic mosaic of human melanoma cells; 2) to attempt to characterize melanoma-associated antigens which are present in the immune complexes (IC) circulating in the serum of melanoma patients. The development of 2) is strictly dependent on the progress achieved in 1).

APPROACH: 1) Attempts are made to develop monoclonal antibodies to surface antigens of human melanoma cells. The homogeneity, together with the specificity and the constant affinity of these antibodies, will be an ideal reagent to characterize in molecular terms the different melanoma-associated antigens. This study may eventually provide valuable reagents which can be of help in the diagnosis of nevus-melanoma borderline lesions. 2) As preparatory study to the development of this point, different biological substrates which have affinity for IC will be assayed as probes for isolation and concentration of these immune reactants.

PROGRESS: 1) Monoclonal antibodies to HLA-DR antigens of the major histocompatibility complex have been obtained. Because these antigens are not expressed on normal melanocytes but have been found on melanoma cultured cells, their presence has been sought on fresh, surgically removed tumors. HLA-DR antigens could be detected in all 14 cases studied although the percent of HLA-DR positive melanoma cells is highly variable. A low positiveness has been found to be associated with highly invasive and massive metastatazing tumors. No correlation has been observed between HLA-DR expression and melanogenesis. Fourteen cases of intradermal nevus lesions have been found to be HLA-DR negative. 2) Staphylococcus aureus Cowan I strain inactivated bacteria have been found to be promising reagents in the development of methods aimed at the isolation and characterization of IC. These microorganisms which bind IC through the antibody Fc portion have been shown to be highly resistant to storage and to strong eluting agents. They rapidly bind IC formed both in vitro and in vivo under conditions of wide molar ratios. The complex bacteria-IC can be used as immunogen for obtaining specific antibodies to the antigen portion of the IC.

BIBLIOGRAPHIC REFERENCES: Isolation of soluble immune complexes from serum using Protein A bearing Staphylococcus aureus bacteria: separation of the antigen from immune complexes and production of antisera. P.G. Natali, L. Walker, M. Pellegrino. Clin. Immun. Immunopath. 15:76, 1980.

312. SEARCH FOR TUMOR-ASSOCIATED ANTIGENS IN HUMAN MELANOMA EXTRACTS

Canevari, S., Della Porta, G., Fossati, G., Miotti, S., Vaglini, M., Cascinelli, N., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To isolate and purify tumor-associated antigens from either melanoma cell lines or fresh melanoma specimens.

APPROACH: Crude soluble material obtained by 3M KCl method from tumor specimens and from crude membrane preparations of surgical specimens and cell lines will be purified by gel-filtration, ultracentrifugal flotation in KBr and polyacrylamide gel electrophoresis. The antigenicity and specificity of the various preparations will be tested by leukocyte adherence inhibition assay, by agarose leukocyte migration inhibition assay and, in some instances, by delayed cutaneous hypersensitivity reaction (DCHR).

PROGRESS: Soluble extracts obtained either directly from melanoma specimens or from crude membrane preparations were assayed by DCHR in patients with malignant melanoma. Both types of preparations gave positive reactions, the reaction of the crude membrane preparations being positive when the amount was ten times smaller.

313. ISOLATION OF TUMOR ANTIGENS OF HUMAN MELANOMA

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The objectives are to continue the purification, isolation and characterization of human melanoma associated antigens (MAA), to study their specificity and the mechanisms of their release by viable tumor cells. If successfully purified, MAA will be used to develop improved assays of humoral immunity to, and circulating antigens associated with, melanoma.

The approach will be to radiolabel macromolecules on the surface of melanoma cells using the lactoperoxidase technique and cytoplasmic proteins with labelled amino acids. Macromolecules will be solubilized by lysis in non-ionic detergents, or if 'shed,' collected in soluble form from culture media. MAA will be identified by double antibody antigen binding assay and radioimmunoassay, utilizing xenogeneic anti-melanoma antibodies exhaustively absorbed with normal tissues. Conventional biochemical procedures and solid phase immunoadsorbents will be used to purify MAA. Specificity will be studied by quantitative absorption, radioimmunoassay and comparison with labelled ma-

cromolecules on control cells. If MAA are successfully purified, several aspects of MAA biology relevant to understanding or modification of tumor growth will be studied, including: a) the immunogenicity of MAA in vivo, and methods of enhancing it, b) the mechanisms of release of MAA by viable cells, c) the effect of MAA release on the susceptibility of tumor cells to immune destruction, d) the utilization of purified MAA for the development of improved assays of antibodies to, and circulating antigens associated with, melanoma.

If successful, these studies may provide tumor antigens useful for the specific immunotherapy of melanoma and for assays of the extent, prognosis and response to therapy of this cancer. Furthermore, we hope to understand, and thus influence, antigen release by tumor cells, a process which may have a profound impact on immune resistance to cancer.

314. MOLECULAR PROFILE OF HUMAN MELANOMA ANTIGENS

Reisfeld, R. A., Morgan, A. C., Galloway, D. R., Quaranta, V., Scripps Clinic & Research Foundation, *476 Prospect St., La Jolla, California*, 92037, United States of America

The major overall objectives of this project are 1) to determine the molecular profile of human melanoma associated antigens with particular emphasis on the chemical structure of their antigenic sites, 2) to elucidate mechanisms of antigen transport, expression and shedding with emphasis on the role played by carbohydrate, and 3) to assess the feasibility of detecting antigens shed from malignant melanoma cells into the circulation which correlate with tumor growth in an animal model system. To attain these major objectives, melanoma associated antigens are isolated and highly purified by immunochemical techniques utilizing monoclonal antibodies. Emphasis is placed on obtaining protease-derived fragments of melanoma associated antigens still retaining their antigenic reactivity and on thoroughly characterizing them by chemical means to assess the molecular nature of their antigenic site(s). In vitro translation of melanoma associated antigens in the absence of glycosylation serves to delineate the role of carbohydrate in affecting antigenic structures while inhibitors of N-glycosylation are used to elucidate the role of carbohydrate in mechanisms governing antigen transport, expression and shedding. It is anticipated that results obtained from the proposed studies will aid in gaining an understanding of the molecular basis underlying functional changes of malignant cells, particularly molecular mechanisms involved in the interaction of tumor cells with the host's immune system. Research designed to determine in an animal model system whether it is possible to correlate tumor growth with the shedding of tumor antigens into the circulation should make it possible to critically assess the utility of such antigenic markers for the detection and prognosis of human cancer.

315. ARE HUMAN MELANOMA ASSOCIATED ANTIGENS MODULATED HISTOCOMPATIBILITY ANTIGENS?

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The major aim of this study is to gain a better understanding of the molecular level of the role played by cell surface molecules in the development of cancer in man and the immune protection against neoplastic disease. To this end, efforts will be made to assess 1) whether human melanoma associated antigens are chemically modulated human histocompatibility antigens, i.e., self-recognition markers, and 2) whether there is any correlation between the chemical structure and the biological function of these tumor associated antigens.

316. IMMUNOCHEMICAL CHARACTERIZATION OF ANTIGENS IN MELANOMA

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Cultured melanoma cells express DR antigens, a set of histocompatibility antigens which have restricted tissue distribution and share several biological molecular properties with murine Ia-E subregion antigens. Xenoantisera to human melanoma associated antigens have been prepared and characterized with serological assays. These xenoantisera have been used to define the biological and molecular profile of human melanoma associated antigens.

317. ANTIGENS OF MALIGNANT MELANOMA AND OTHER HUMAN TUMORS

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Glycoproteins are important constituents of the cell surface of all mammalian cells, both normal and abnormal, many of which serve as antigens in autologous, syngeneic and xenogeneic immunizations. They have been implicated in many other properties of cells which involve specific recognition and interactions. The main objective of this study is to analyze the surface glycoproteins of cultured cells from malignant melanoma and other human tumors. One aspect which will be studied is the apparent high degree of sialylation exhibited by the cell surface glycoproteins of melanoma. This is in contrast to other tumor and normal cells studied and may be related to the biological characteristics of melanoma. The remainder of the study will emphasize two antigens of melanoma and other tumors: Gp110 and DR or Ia-like antigens. The former, which is detected by a specific rabbit antiserum, appears to be the major glycoprotein of a number of cell types, both normal and malignant. Its significance lies in the possibility that it may inhibit structural differences in its carbohydrate chains in malignant as compared to normal cells. Ia antigens were originally considered to be confined to cells of the hematopoietic system but have recently been found to occur on other cells, including melanoma. Several aspects of the expression of Ia in tumors will be studied: (1) quantitation of Ia antigen in different melanoma lines, in melanoma tumors and in other tissues and cells, both normal and abnormal; (2) structural characteristics of melanoma Ia antigen; and (3) comparison of melanoma Ia with lymphocyte Ia using cells derived from the same individual. The significance of Ia expression in melanoma lies in its possible role as a differentiation antigen and in influencing the immune response to the tumor.

BIBLIOGRAPHIC REFERENCES: R.J. Winchester, C-Y. Wang, A. Gibofsky, H.G. Kunkel, K.O. Lloyd and L.J. Old. Expression of Ia-like antigens on cultured human malignant melanoma cell lines. Proc. Nat. Acad. Sci. 75:6235 (1978).

318. THE MOLECULAR NATURE OF TUMOR ANTIGENS

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Our group has detected three potentially interesting cell surface antigens which appear to be tumor associated. The antigens were initially detected on human melanoma cells using specific monoclonal antibodies. Extensive testing has shown that one of the antigens (3.1) has an extremely restricted distribution. The chemical nature of this antigen has not as yet been determined. The other two melanoma-associated antigens are proteins with molecular weights of approximately 97,000 (p97) and 210,000 (p210), respectively. These two protein antigens have similar types of distribution in that each is present in many of the melanoma cell lines and in some of the non-melanoma tumor cell lines tested, but not in any of the normal cell lines tested. Many normal adult human tissues have been examined for the presence of p97 and p210 and to date neither protein has been detected in these preparations. Both p97 and p210 are now being purified by immunoadsorption procedures using monoclonal antibodies. We propose to determine the chemical and structural natures of the purified antigens. Radioimmunoassays will be developed in order to quickly screen for these antigens in normal and malignant tissues as well as in sera from cancer patients.

BIBLIOGRAPHIC REFERENCES: Woodbury, R.G., Brown, J.P., Yeh, M-Y., Hellstrom, I. and Hellstrom, K.E. Identification of a cell surface protein, p97, in human melanomas and certain other neoplasms. (1980) Proc. Natl. Acad. Sc., USA. 77:2183-2186. Brown, J.P., Wright, P.W., Hart, C.E., Woodbury, R.G., Hellstrom, K.E., and Hellstrom, I. Protein antigens of normal and malignant human cells identified by immunoprecipitation with monoclonal antibodies (1980) J. Biol. Chem. In press.

319. CHANGES IN THE ANTIGEN STRUCTURE OF MOUSE EPIDERMIS DURING CARCINOGENESIS

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OBJECTIVE: To determine whether painting of hairless mouse skin with carcinogens leads to deletion of some of the epidermal antigens, and possible occurrence of new antigens.

APPROACH: Antibodies are produced by injection of homogenized epidermis, hyperplastic epidermis during carcinogenesis (papillomas and carcinomas) into rabbits. Immunological analysis is made by the gel diffusion precipitation line technique of Ouchterlony.

PROGRESS: It has been observed that during carcinogenesis, the epidermis loses some of its specific antigens, and acquires one new antigen which is also found in normal thymus and spleen, and probably comes from lymphocytes which are always present in papillomas and carcinomas.

320. SWINE MELANOMA ANTIGENS - ISOLATION AND EVALUATION

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The swine melanoma tumor system is an animal model of spontaneous malignant disease that has many features in common with its human counterpart. Features of this tumor system include a histopathologic resemblance to human superficial spreading melanoma, a high incidence of primary cutaneous melanomas, a high incidence of benign melanocytic lesions that transform to malignant melanoma, and the occurrence of metastatic disease. The objective of this project is to isolate and partially purify melanoma-associated antigen(s) from primary cutaneous melanomas and metastatic lesions and to compare these antigens with similarly isolated fractions from benign melanocytic lesions and normal black swine skin. Two approaches will be used to isolate antigenic fractions from 3M KCl tumor extracts. A biochemical approach will utilize a combination of techniques, including gradient density centrifugation, polyacrylamide gel electrophoresis, and anion exchange chromatography. An immunologic approach will utilize affinity chromatography techniques both to remove normal tissue antigens and to isolate melanoma-associated antigen(s) from tumor extracts. Fractions from various tumors and different tumor types will be evaluated for melanoma-associated antigen activity and immunologic cross-reactivity. Thus, this unique animal model provides the opportunity to evaluate the relationship between melanoma-associated antigens and the cellular events associated with neoplastic transformation and progressive or regressive growth.

321. TUMOR REJECTION ANTIGENS FROM TREATED CANCER CELLS

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The purpose of this investigation is to solubilize, purify, isolate and characterize tumor rejection antigens from immunogenic mouse melanoma and mammary adenocarcinoma cells. The melanoma cells were rendered nontumorigenic and immunogenic by long-term culture in 1 microgram/ml of bromodeoxyuridine, and the mammary tumor cells by long-term culture in normal medium. Plasma membranes will be isolated by differential centrifugation from cells disrupted by nitrogen cavitation and fractionated on sucrose-Dextran and sucrose flotation gradients. Characteristics of the purified subcellular fractions will be monitored by radioimmune and biochemical assays. Immunogenicty will be tested both by in vivo tumor rejection assays and by in vitro assays worked out with whole cells. Soluble fractions will be prepared from the immunogenic fraction and purified by apropriate chromatographic methods. Immunochemical analysis of the membrane fractions will be carried out using antisera we have prepared. Biochemical studies on the glycosaminoglycans and proteoglycans of these cells will be made and correlated with their unusual biological properties. The ultimate aim of these studies is to use them as model systems forming a basis for safe immunization of melanoma and breast cancer patients after removal of their primary tumors.

322. ISOLATION OF SOLUBLE PURIFIED TUMOR-ASSOCIATED ANTIGENS OF HUMAN MALIGNANT MELANOMA

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OBJECTIVE: Preparation of soluble purified tumor-associated antigens of human malignant melanoma that can be used for tests of cell-mediated and humoral immunity and for the determination of circulating tumor-associated antigens in melanoma patients.

APPROACH: Mainly tissue and membrane preparations from excision-biopsies of human malignant melanoma will be used for the extraction of tumor-associated antigens. Smaller preparations are planned from long-term cell lines of malignant melanoma. The extracts will be purified by salt precipitation, ion-exchange chromatography, and gel electrophoresis. Antigenic activity will be monitored with heteroantisera of rabbits and monkeys by the following assay methods: 1) immune precipitation; 2) passive hemagglutination; 3) radioimmunoassays; and 4) counter-current electrophoresis.

Purified preparations of antigens will be characterized by quantitative determinations of neutral sugar, amino sugar, and protein.

The occurrence of the isolated and characterized antigens in normal and inflammatory tissue, in embryos, and in different malignancies will be tested.

The clinical relevance of the isolated antigens will be determined by comparison of individual in vitro results of immunity tests with the clinical course of the patients. Leucocyte migration assay, adsorption of cytotoxic lymphocytes by immunoadsorbents prepared with isolated antigens, and determination of circulating antigen levels by radioimmunoassays are planned.

PROGRESS: Antigen was extracted with the non-ionic detergent NP-40 from membranes of 3H-leucine labelled cultured melanoma cells. Antigenic activity was assayed with sera from melanoma patients.

323. TUMOR ANTIGENS AND ANALYSIS OF THE IMMUNE RESPONSE

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OBJECTIVE: To isolate and characterize tumor associated antigens from surgical specimens of tumoral tissues of different histologic type. The antigenicity of various extracts will be assayed by tests of cellular immunity.

APPROACH: KCl extracts from surgical specimens will be further purified by ultracentrifugal flotation on KBr, gel-filtration and affinity chromatography. The different extracts will be analyzed by gel electrophoresis in polyacrylamide. The immunogenicity and the specificity of the extracts will be assayed by delayed cutaneous hypersensitivity reaction (DCHR) and by two in vitro tests of cellular immunity (LAI and LMI).

PROGRESS: Removal of lipoproteins, HLA and human serum albumin from a negative KCl extract of kidney carcinoma gave a steady increase in positive DCHR. This reactivity seemed to have a certain degree of tumor and histologically associated specificity. Further study performed in melanoma patients did not confirm the results obtained in kidney cancer. In fact we observed an increase in frequency of reactivity with purification but this reactivity seemed not to be histologically associated. In addition a variability in the reactivity was observed among different melanoma extracts.

324. TUMOR ASSOCIATED ANTIGENS AND IMMUNOGLOBULINS IN MAN

Dorval, G., Schulz, J., Wilkinson, R. D., Osterland, C. K., Shibata, H. R., Royal Victoria Hospital, Clinical Immunology Division, *687 Pine Ave. W., Zone 2, Montreal, Quebec, Canada*

OBJECTIVES: Isolation of melanoma neoantigens (TAA) from malignant effusions and from circulating immune complexes (IC). Identification of melanoma-associated antibodies (T-Ig) within sera and isolated IC from malignant melanoma patients and from individuals with vitiligo. Production of melanoma specific xenoantisera. Analysis of the dynamics of 'normal'

versus 'malignant' surface antigenic components on melanoma cells. Immunologic monitoring of disease/treatment activity.

APPROACH: We will isolate melanoma-type specific neoantigens and proceed to molecular characterization of purified components with ascertained reactivity for T-Ig. Xenoantisera will be raised against 'promising' TAA samples and will be used for monitoring further the purification of TAA. Extensive adsorption of antisera will be done using normal and malignant tissue/cell lines. Specificity of the antisera will be assessed by inhibition of cytotoxicity of melanoma cells by different tumor extracts. Anti-TAA xenosera will then be used to study the turn-over of melanoma membrane components.

PROGRESS: We have now developed a micro-radioimmunometric assay capable of detecting 10 to the minus 12th power g of IC (modified from Yang et al., J. All. Clin. Immunol. 62:300, 1979). K-562 cells with 'blocked Fc-receptors' (using aggregated IgG) (HAGG) are first reacted with sera/fluids potentially containing T-Ig/TAA to remove any K-562 antibody activity. Sera and effusions are also adsorbed onto pooled, immunolized immunoglobulins and lymphocytes. They are then reacted in different ratios and incubated with 'fresh' K-562, which has a high density of Fc-receptors. Adsorption of IC is quantitated by radiolabeled S. aureus protein A. Using criss-cross specificity controls with non-malignant sera/effusions, T-Ig could be detected in 21/34 stage I melanoma patients, against a 300,000 d. fraction from a melanoma malignant effusion, but not against any other sera/effusions thus far tested.

325. MAINTENANCE OF HUMAN TUMORS IN VITRO FOR IMMUNOLOGICAL STUDIES

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The original objectives of this ongoing project were to validate the use of human cancer cell lines for the study of tumor antigens and immune responses to these antigens using malignant melanoma as the model. To this end, much progress has been made in the development and maintenance of well-defined, mycoplasma-free melanoma lines, and in the search for antitumor immune responses in patients, as well as in the raising of xenogeneic antisera to characterize tumor-associated antigens present on those lines. Continuing work will be directed along the following lines: A) Maintenance of cell stocks and establishment of new cell lines which entails characterization using a number of parameters and, in particular, monitoring for mycoplasma. B) Development of highly antigenic sublines. Xenogeneic antibodies have revealed that some cell lines are consistently more reactive than others suggesting that melanoma associated antigens (MAAs) may be expressed to a different degree on different cell lines. Highly reactive clones will be isolated, and after determining whether this high reactivity is stable we will evolve a panel of such cell lines to define the spectrum of MAA polymorphism as revealed by xenoantibody. This panel will then be evaluated in immunological tests using human sera to determine if the same MAAs are recognized by and are relevant to man. C) Use of selected tumor cells for immunotherapy of melanoma. The use of specific active immunotherapy in melanoma has not been adequately evaluated in terms of potential benefit and in terms of the clinical relevance of the antitumor immune responses which can be measured in such patients. We plan to carry out a preliminary nonrandomized trial of patients with recurrent melanoma to determine if the induction of specific antimelanoma antibodies can be correlated with an improved survival.

326. HL-A IN FAMILIAL MALIGNANT MELANOMA - GENETICS OF SKIN CANCER

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The following investigations are in progress: (1) Studies of HL-A in six families in which more than one member has had malignant melanoma have been very rewarding. It is proposed to continue this study in a further sixteen families and hence possibly further define a sub-group-at-risk for malignant melanoma. (2) A genetic diathesis termed 'Celticity' has been established in skin cancer in Australians and in residents of Northern America. HL-A phenotyping as well as other genetic markers are being used in early severe cases of basal cell and squamous cell carcinoma and the role of other factors such as wind, infrared and ultraviolet exposure and humidity are being investigated. Controls consist of age and sex matched patients without skin cancer.

327. IMMUNODIAGNOSIS OF MELANOMA

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The primary objective of this research is the development of a sensitive radioimmunoassay (RIA) for the detection of human melanoma tumor associated antigens (TAA). This assay would be useful for monitoring the course of melanoma patients undergoing various forms of therapy and for the investigation of the roles of soluble TAA and TAA-antibody complexes in the disease process. Operationally monospecific alloantisera and autoantisera from melanoma patients and chimpanzee, monkey and rabbit heteroantisera against melanoma TAA are being employed to isolate pronase-solubilized melanoma TAA and serum-borne TAA. These, in turn, will be used to produce highly specific heteroantisera for use in the RIA. Serum and urine of melanoma patients will be compared to those of control donors to validate the RIA.

In vitro and in vivo antibody localization procedures are also being developed using autoradiography and immunofluorescence. Human melanomas growing in nude mice and the B16 melanoma of C57BL/6 mice are being used as animal models to further these studies. Passive serotherapy will then be attempted in these models.

The antimelanoma antisera will also be used for solid tumor immunodiagnosis where diagnosis cannot be made using conventional pathology techniques.

328. CELL SURFACE ANTIGENS OF HUMAN MELANOMA IDENTIFIED BY MONOCLONAL ANTIBODY

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We have recently shown (Yeh et al., PNAS, in press) that the Milstein hybridoma technique can be used to raise monoclonal antibody to an antigen present on a human melanoma. We want to characterize this antigen, and to use the Milstein technique for raising hybridomas with specificity for other antigens unique to one human melanoma, or shared by such tumors. For this purpose, we have five specific aims: 1) To characterize further the three melanoma-specific hybridomas isolated (3.1, 3.2, and 3.3), using high titered antibodies produced in hybridoma-bearing mice. The antibodies will be tested for specificity by absorption techniques, binding assays (including membrane immunofluorescence) and complement-dependent cytotoxicity, and their abilities to function as lymphocyte-dependent antibodies will be investigated. 2) To establish new hybridomas, some producing antibodies to unique antigens, and some to antigens shared by many (or all) melanomas, and to test them as outlined under Aim 1. 3) To relate antigens detected by using the Milstein hybridoma technique to antigens defined by sera from the autochthonous tumor-bearing patient, and to determine the expression in vivo (in surgical biopsies and in tumors growing in nude mice) of the melanoma antigens detected on cultivated tumor cells. 4) To determine whether the melanoma antigens undergo modulation in the presence of specific antibody and whether variant melanoma cells that do not express the antigens can be isolated after exposure of the cells to antibody and complement. 5) To purify and characterize biochemically the melanoma antigens defined by the hybridoma antibodies and develop competition radioimmunoassays.

We believe that these experiments will provide information of direct relevance to the diagnosis and treatment of human melanoma.

329. SEROLOGIC ASSAYS WITH MELANOMA ASSOCIATED ANTIGENS

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OBJECTIVES: Isolate and characterize cell surface antigens of human malignant melanoma and develop a sensitive serologic assay for the monitoring and diagnosis of this disease.

330. HUMAN MELANOMA AND TUMOR SPECIFIC MONOCLONAL ANTIBODIES

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The proposed studies will lead to the production of a panel of monoclonal mouse and human melanoma antigen epitope specific antibodies. Antigens with which these antibodies react will be isolated and characterized. Studies will provide information on antigenic changes which accompany tumor progression. The clonal nature (or otherwise) of metastatic tumors will be established. The antigenic relationships of benign, premalignant and malignant human melanotic tissues will be examined and the role of the human immune response to melanoma antigens will be defined. Monoclonal tumor specific antibodies provide an unparalleled opportunity to study the antigens and immune responses to human melanoma and will allow the development of a rational approach to human tumor diagnosis and immunotherapy.

331. LYMPHOCYTE SENSITIZATION TO TUMOR ANTIGENS IN VITRO

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Several areas of continued research on the sensitization of human lymphoid cells to antigens on melanoma cells are proposed. Lymphocyte subpopulations will be tested for their ability to be specifically sensitized to autochthonous melanoma cells, and studied with regard to the expression of natural killer (NK) cytotoxicity. A system of T-cell 'help' has been investigated, and will now be applied in an attempt to augment the development of cytotoxic effector cells. Secondary sensitization with soluble tumor antigens after in vivo or in vitro priming will be investigated. The sensitization system will be used to study the expression of various melanoma-associated antigens and histocompatibility restrictions on the cytotoxic response.

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332. TRANSPLANTATION ANTIGENICITY OF VIRUS INDUCED TUMORS

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We are continuing our work on humoral and cell-mediated immune reactions to human tumor-associated antigens. A major effort at this time goes into using the monoclonal antibody technique to detect and characterize cell surface antigens of various human tumors, particularly melanomas. So far, antigens of very restricted distribution, as well as antigens shared by many tumors, have been detected. A particularly interesting antigen, p97, has been demonstrated in about 90% of human melanomas and about 50% of other human tumors, but has not, so far, been detected in normal human tissues. We are attempting to develop radioimmunoassays for this antigen, to study its distribution further, to investigate to what extent it is expressed in vivo, whether it can undergo antigenic modulation, and whether a p97 positive tumor gives rise to variants which lack p97. Similar studies are being conducted with p210, another human tumor-associated antigen, which we recently identified: p210, has, so far, been less studied for specificity. Several other hybridomas forming antibodies reacting with cell surface components of human tumor cells, are presently being worked up. The relationship, if any, between antigens detected by the hybridoma technique, such as p97, and antigens recognized by the patients (in the form of targets for humoral or cell-mediated reactivity) will be investigated, using various blocking assays.

Other studies concern the targets of cell-mediated immune responses of Moloney virus-induced sarcomas, as outlined in my original grant application.

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333. EXPERIMENTAL THERAPY OF HUMAN MELANOMA WITH VACCINES

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While the knowledge of microbial antigens has permitted the successful development of vaccines against infections, lack of comparable knowledge of human cancer antigens has hampered the development of human cancer vaccines of defined cancer-restricted antigenicity. Recent advances based on autologous serological typing of cultured melanoma cells have led to the identification of two classes of melanoma-restricted cell surface antigens, Class I, detected only on the autologous melanoma cell, and Class II, shared by other melanomas. We propose to test, in a sequential fashion, various types of melanoma vaccines expressing Class I/II antigens to determine conditions for maximal immunogenicity. In initial tests of vaccines prepared from irradiated autologous cultured melanoma cells, antibody to Class I/II antigens was rarely induced or augmented. Therefore, we intend to test vaccines carrying additional antigenic determinants, with the expectation that these determinants will provide 'help' for the recognition of Class I/II melanoma antigens. The vaccines will be prepared from (1) irradiated cultured allogeneic melanoma cells expressing cross-reacting melanoma cell surface antigens, (2) irradiated cultured autologous melanoma cells infected with vesicular stomatitis virus, (3) irradiated chemically modified autologous melanoma cells, (4) irradiated intraspecies or interspecies hybrids expressing Class I/II antigens, and (5) immunogenic forms of isolated Class I/II antigens. The relevant immunogenicity of the vaccines will be determined by comprehensive analysis of the patients' antibody response to Class I/II antigens. This is an experimental pilot study concerned with analysis of the serological response to vaccine administration. If a change in serological reactivity to Class I/II melanoma antigens is induced with some regularity, a clinical immunotherapy trial will be designed.

334. HUMORAL AND CELLULAR IMMUNITY AGAINST SURFACE ANTIGENS OF MALIGNANT MELANOBLASTOMA CELLS

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OBJECTIVE: The study of hosts' immunological factors for better diagnosis and treatment of malignant tumors.

APPROACH: Detection, isolation and characterization of immune complexes (IC) at the tumor level and in the circulation. A C1q-ELISA and polymorphonuclear (PMN) phagocytosis test are used for detection of IC. Further isolation and characterization with affinity chromatography with protein A and anti-IgG rheumatoid factor. Search for melanoma antigens in IC with the use of purified antisera. These are obtained from patients and from immunized rabbits. Specificity is tested with an in situ membrane immunofluorescence method on monolayers of cultured tumor cells. These are used as living cells whereas in a recent modification a paraformaldehyde prefixation is being applied. For purification autologous transformed B cells are used in the final absorptions. Finally, melanoma antigens in tumor fractions are studied by in vitro stimulation tests of autologous lymphocytes. Reactivity is measured by 3H-thymidine incorporation in cellular DNA and 3H-uridine in RNA.

PROGRESS: A relationship between the level of in vivo phagocytosed IC staging and tumor burden was found. A sig-

nificant drop in IC after tumor surgery was seen in patients with favorable prognosis (T.H. The et al., Clin. Exp. Immunol. 32, 387, 1978). Results of the study of IC at the tumor level are described in the proceedings of the Colloquium 'Protides of the Biological Fluids' 1-5 May, 1978. For the detection of IC a new technique was developed: the C1q-ELISA. Details of the test will be published.

335. ROLE OF SOLUBLE TUMOR ANTIGEN AND IMMUNE COMPLEXES IN CANCER

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It is widely accepted that tumors express recognizable new antigens on their plasma membrane and that these antigens evoke an immune response in the tumor host. Evidence from our own work and that of others suggests that tumor neoantigens are also present in a soluble form in the fluid compartments of the tumor host. If serum levels of soluble tumor antigens per se or a product of their interaction with the host immune response (namely, soluble immune complexes) can be shown to relate in a predictable fashion to the presence and progress of cancer, their measurement by sensitive radioimmunoassays is potentially the most economical, specific and widely applicable method for diagnosis and subsequent prognosis of cancer. Our concept of the tumor-host immunologic relationship also suggests that new methods of 'immunotherapy' of cancer might profitably be directed at reduction of circulating soluble antigens and/or immune complex level.

The immediate objective of the proposed research is to thoroughly evaluate in a well-defined murine melanoma model the diagnostic and prognostic potential of measurement of serum levels of soluble tumor antigen and soluble immune complexes and the therapeutic potential of various methods of altering the levels of these entities in the tumor host.

The ultimate objective is to devise similar diagnostic and/or prognostic assays and therapeutic modalities for human cancer patients. The initial steps toward this objective will be taken in the proposed study in the following areas: 1) Evaluation by a new method developed by us of serum immune complex levels in relation to tumor presence and progress in selected cancer patients; 2) application of a new approach to the isolation of soluble human tumor antigens.

336. RADIOIMMUNOASSAY FOR MELANOMA ASSOCIATED ANTIGEN

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This is part of a broader project. A summary of this subproject is not available.

F. ANTIGENS OF GASTROINTESTINAL, LIVER, AND PANCREATIC CANCERS

337. IMMUNOLOGICAL STUDIES ON GASTROINTESTINAL TUMORS

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OBJECTIVES: (1) To isolate and characterize tumor antigens using patients' antibodies as specific reagents; (2) to assay the antibody production of patients; (3) to determine circulating tumor antigens from patients; (4) to assay immune complexes in patients; (5) to evaluate the diagnostic and prognostic value of blood tests for antigens, antibodies and immune complexes.

APPROACH: Solid phase radioimmunoassays have been developed for the determination of tumor antigens and antibodies. For the detection of tumor antigens, specific antibodies are linked to cyanogen bromide activated paper discs and used for the separation of antigens from serum; the antigens are detected with 125I-labelled tumor antibodies. For the assay of antibodies 3M KCl tumor and normal extracts are linked to the discs which fix tumor antibodies that are detected with radiolabelled antiglobulins. Sodium dodecyl sulfate polyacrylamide gel electrophoresis is used for the characterization of tumor antigens in combination with physico- and immunochemical methods.

PROGRESS: A significant proportion of patients (30-40%) produces tumor antibodies which may belong to the IgA, IgE, IgG or IgM classes. (No tests were performed for IgD to date). During electrophoresis patients' antibodies (double antibody technique) identify distinct peaks from tumor extracts, not present in normal tissue extracts or in CEA.

338. PURIFICATION AND CHARACTERIZATION OF TISSUE ANTIGENS OF THE GI TRACT

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The objectives of the project are to extend our knowledge of the nature and etiopathogenesis of chronic atrophic gastritis (CAG) which is the basic lesion in pernicious anemia (PA) but may occur in association with gastric ulcer and gastric carcinoma without the lesion of PA. The research plan continues to be the isolation and purification of gastric cellular antigens against which humoral and possibly cell-mediated immune responses are directed in PA but not in all forms of CAG.

Human gastric juice and preparations of fetal and adult human gastric mucosa are being processed by such techniques as affinity chromatography and density gradient ultracentrifugation in attempts to produce antigenically pure materials against which 1) antibodies can be produced in immunized animals, 2) circulating white cells from PA patients and controls can be tested by techniques such as agarose-leucocyte migration for evidence of sensitization.

To date definitive results have not been achieved because of the very real difficulties inherent in purifying large molecules, especially lipoproteins, which we believe some of our antigens to be. However, we have developed immune techniques to a satisfactory level of reliability by applying them in collaborative studies in preparation for application in our central projects when antigen isolation has been satisfactorily achieved.

The relationship of the project to human disease, including pre-malignant disease of the stomach, is evident.

339. IMMUNOLOGICAL MARKERS OF TUMOR TYPE SPECIFIC ANTIGENS IN GASTROINTESTINAL CANCER

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OBJECTIVE: The continuing purpose of this project is to look for gastrointestinal cancer specific responses in leukocyte adherence, inhibition, blockade or arming thereof. The assay consists of a modification of Thomson's tube LAI method. The applicability of such responses for tumor diagnosis will be evaluated.

RECENT PROGRESS: Five extracts of human colon carcinoma and three 'nonspecific' (genitourinary) human tumor extracts were used to determine percent non-adherence of peripheral leukocytes from individuals in the following categories: 1) colon cancer patients, 2) neoplasms other than colon carcinoma, 3) non-cancer patients, 4) healthy adults.

Percent non-adherence of PPD-positive and PPD-negative adult's peripheral leukocytes were determined, utilizing preservative free tuberculin as inhibitor antigen.

Evaluation and expansion of these experiments are in process.

340. EXPRESSION OF COLON ANTIGENS (WZ) IN HUMAN CANCER CELLS GROWN IN CULTURE AND IN THE CORRESPONDING TUMORS DEVELOPED IN NUDE MICE (ABBREV)

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OBJECTIVE: To study the possible differences between the normal colon polymorphic antigens (WZ) in colon carcinoma cell lines grown in culture and in the corresponding tumors grown in nude mice.

APPROACH: The presence of the WZ antigens in cells and tumors will be assayed by two different approaches. The direct approach will be studying the presence of the WZ anti-

gens in cells and tumors, using three different methods: the immunofluorescence technique, a cytotoxicity test, and radioimmunoassay. The indirect approach will involve immunizing rabbits with cells and tumor extracts. The antisera will be assayed with the same techniques on normal colons and colon extracts for the presence of anti-WZ antibodies. The results will be compared to those obtained for ABH and HLA antigens.

PROGRESS: The definition and technical approach to the study of the WZ antigens in the normal human colon have been published (Zweibaum et al. Tissue Antigens, 1975, 6, 121. Oriol et al. Immunology, 1977, 32, 131. Zweibaum et al., Ann. Immunol., 1977, 128C, 87). Preliminary results obtained in two colon carcinoma cells lines (HT29 and HRT18) and in the corresponding tumors developed in nude mice have shown that the WZ antigens were absent from the cultured cells, but normally present in the tumors.

341. A STUDY OF THE NATURAL HISTORY OF COLORECTAL CANCER - CLINICAL STUDY OF TUMOR MARKERS IN COLORECTAL CANCER

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The summary for this project is under entry no. 5.

342. A MODEL FOR COLONIC CANCER-ASSOCIATED ANTIGENS

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Our overall objective is to identify, isolate, purify and characterize tumor-associated and organ-associated antigens of human colonic tumors. The model developed as a source of such antigens is xenografted human colonic carcinomas, utilizing both hamsters and nude (athymic) mice, as well as cell cultures derived therefrom. Since these experiences with human tumor systems have been found to produce a number of human tumor-associated and organ-associated antigens (CEA, CSA's and CSAp), these systems will be explored further for the presence of other antigens which might be of additional immunodiagnostic or immunotherapeutic importance. However, the major emphasis of this project is the further elucidation of the immunology, immunochemistry, and immunocytochemistry of two new antigen systems described by us in our previous work: colon-specific antigens (CSA's) and colon-specific antigen-protein (CSAp). The latter antigen (CSAp) has been found to be quantitatively increased in rapidly proliferating gastrointestinal tissues, especially colonic polyps and carcinomas, and has also been found to circulate in the blood of patients with colonic cancer. Hence, our immediate concern is the further isolation, purification, and chemical characterization of CSAp. We are also interested in the growth behavior and phenotypic expression of colonic carcinomas and polyps in hamsters and nude mice.

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343. SELECTIVE RADIOIMMUNOTHERAPY OF COLON CANCER

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The summary for this project is under entry no. 16.

344. PURIFICATION OF HUMAN TUMOR ASSOCIATED ANTIGENS

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OBJECTIVES: Extract and purify colonic mucoprotein antigen (CMA) from normal colon and adenocarcinomas of colonic origin for immunologic and chemical analysis. Develop a radioimmunoassay and immunohistochemical detection procedures for CMA. Investigate the ability of antisera to differentiate between normal and tumor CMA to investigate the potential of CMA as a tissue specific or tumor specific marker. Investigate the oligosaccharide and peptide primary structure of CMA. Analyze the relationship of CMA to blood group substance, colon specific antigen, and carcinoembryonic antigen.

345. NUCLEAR ANTIGENS IN HUMAN COLORECTAL CANCER

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Nuclear, nucleolar and chromosomal protein antigens and their complexes with nucleic acids will be investigated in normal and malignant cells of human colon. The antigens will be isolated and extensively characterized in several tissue cultured human colonic carcinoma cell lines as well as in normal colonic mucosal specimens obtained by surgery. Antigens specific for normal or malignant cells will be studied in terms of their relationships to the DNA in transcriptionally active, inactive, replicating and DNA repair undergoing chromatin. Cell-specific antigens will be localized by conjugated antibodies method (fluorescent or peroxidase-antiperoxidase) in an attempt to develop histoimmunochemical methods for the detection of malignant and possible pre-malignant cells in frozen sections of human colon. Syrian hamsters will be employed in experiments on molecular mechanisms leading to qualitative changes (detectable immunologically) of nuclear proteins during experimental colorectal carcinogenesis. Attempts will be made to correlate the genomic distribution of the cancer-associated antigenic sites with the interaction sites of chemical carcinogens.

346. ANTIGENIC STIMULUS IN HUMAN COLON CARCINOMA IMMUNITY

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Human colon cancer is one of the most common malignancies in this country. The proposed investigation seeks to chemically and immunologically characterize cell surface antigens distinctive of colonic neoplasia signals and receptor sites determining organoid differentiation. The applicants have previously initiated, established and characterized human colon cancer cell lines in continuous tissue culture. Chemical studies comparing the surface membrane profiles as well as solubilized components of neoplastic versus normal colonic tissue cells revealed multiple differences. Immunological tools are being used to determine which of the altered proteins is distinctive of the neoplastic state and might be harnessed as immunodiagnostic and/or immunotherapeutic tools. Monoclonal anti-colonic carcinoma antibodies prouced by hybridization of myeloma (P3 x 63AG8) with lymphoid cells derived from murine hosts pre-immunized with whole cell or soluble extracts of a variety of colonic neoplastic cell lines will be tested for a) immunological reactivity detected by radioproline cytotoxicity assays and by immunoglobulin binding detected with SPA or antiglobulin against the battery of colonic and other neoplastic cell lines, as well as normal human lymphocytes, colonic adult and fetal normal epithelium in continuous culture; and b) susceptibility to inhibition upon preincubation with purified antigenic fractions. Normal colonic epithelial cell cultures will be established utilizing a combination of new isolation methods and application of proliferative stimulus growth factors, insulin, hydrocortisone, polyamines, and gastrin, as indicated in our previous work with colonic and urothelial cells. Colonic cell extracts solubilized 3M KCl extraction and/or a butanol extraction are fractionated by preparative isoelectric focusing, lectin affinity chromatography and two-dimensional polyacrylamide gel electrophoresis. Further colon cancer lines will be utilized to dissect factors responsible for organoid differentiation of neoplastic cells, utilizing the model of glandular formation on hollow fiber by some cultured cells and their clones. These studies may provide insight into humoral mediators which might be developed as chemotherapeutic agents to enhance organoid formation and possibly retard neoplastic proliferation of anaplastic tumors. These fundamental studies may afford insight into distinctive neoplastic antigens capable of triggering immune recognition.

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347. MOLECULAR APPROACHES TO HUMAN COLON CANCER

Kahan, B. D., Tom, B. H., Rutzky, L. P., University of Texas, Health Science Center at Houston, School of Medicine, Dept. of Surgery, *Box 20036, Houston, Texas*, 77025, United States of America

While a variety of histiotype-specific tumor antigens have been identified on some human neoplasms, e.g., malignant melanoma, and tumor-specific antibodies readily produced, human colon cancer, the most common neoplasm in this country, has been refractory to such an analysis. There is no unequivocal evidence of tumor-specific antigens on human colorectal cancer cells, although common, tumor-specific antigens have been demonstrated on chemically induced rat colon carcinomas. This application proposes to harness immunological and chemical tools to dissect cell surface components mediating organoid differentiation, inter-cellular adhesion, and tumor specific antigenicity. Cell surface markers will be dissected on human colon cancer cell lines (LS174, LS180 and LS123) as well as their clonal variants, which have been characterized morphologically and by in vitro and in vivo (tumorigenicity) growth. Factors controlling glandular, organoid differentiation, as displayed in hollow fiber culture systems, are dissected by admixing materials derived from appropriate colon cell lines and clones to lines and/or clones which are incapable of displaying this organization. Mediators of organoid differentiation are identified by addition of supernates and/or membrane fractions to the extracapillary compartments of hollow fibers. Inter-cellular adhesive factors are identified by reaggregation in spinner cultures and by formation of multicellular tumor spheroids. Antigenic surface structures distinctive of malignancy are dissected utilizing antibodies, which have already been produced by hybridomas of murine myeloma cell lines with spleen cells from mice immunized with neoplastic lines. Specificity of the monoclonal xenoantibodies is monitored by reactions against normal adult and fetal colonic mucosal cells, normal cells from other tissues, and cancer cells from other neoplasms, particularly those producing CEA. These studies may elucidate cell surface structures critical to the expression of the neoplastic state, which might serve as a focus of immunotherapy.

348. PREPARATION OF AN ORIGINAL RIA METHOD FOR DETERMINING CEA OF ADENOCARCINOMA OF THE COLON IN PLASMA

Buraggi, G. L., Bombardieri, E., Ringhini, R., Rodari, A., Turrin, A., Clerici, L., Istituto Nazionale per lo Studio e la Cura dei Tumori, Dept. of Nuclear Medicine, *1 Via G Venezian, 20133 Milan, Italy*

The summary for this project is under entry no. 12.

349. STUDIES OF TUMOR ASSOCIATED ANTIGEN RELATED TO COLON CARCINOMA

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No summary has been provided to the Smithsonian Science Information Exchange.

350. HEPATITIS B SURFACE ANTIGEN (HBSAG) IN LIVER DISEASES FROM DIFFERENT GEOGRAPHICAL AREAS

Nayak, N. C., Cuello, C., Mandard, A. M., Quenum, C., Munoz, N., All India Inst. of Medical Sciences, Dept. of Pathology, *Ansari Nagar, New Delhi 110016, Delhi, India*

Six hundred seventy-two postmortem liver specimens from patients with primary liver cancer, cirrhosis and other liver diseases, from high, intermediate, and low risk areas for liver cancer, have been examined for hepatitis B surface antigen using the Shikata orcein stain. The association between hepatitis B and primary liver cancer shown in serological studies is confirmed in these tissue studies, and, in addition, a positive association between this virus and non-alcoholic cirrhosis is observed in all geographical areas, but no association is observed for alcoholic cirrhosis.

351. ECTOPIC ANTIGENS IN HEPATOCELLULAR CANCER

Higgins, P. J., Sloan Kettering Inst. for Cancer Research, Laboratory of Cell Biochemistry, *410 E. 68th St., New York, New York*, 10021, United States of America

Comparative in vitro and in vivo studies of the antigenic composition and growth properties of rodent cells derived from normal and neoplastic adult liver tissue will be carried out. The occurrence of fetal and ectopic antigen expression, as determined by immunochemical methods, will be correlated with the degree of hepatocellular pathology observed and the in vitro growth properties of liver-derived cells. The usefulness of the expression of a specific ectopic antigen (gamma-fetal antigen) as an immunodiagnostic antigen will be evaluated. Experiments concerning the potential modulation of ectopic antigen expression by the IgG fraction of anti-gamma-fetal antigen serum, or the Fab fragments prepared therefrom, may elucidate specific associations between ectopic antigen production and the process of neoplastic transformation.

352. SURFACE ANTIGENS OF RAT HEPATOCELLULAR CARCINOMAS

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Malignant transformation is accompanied by extensive cell-surface alterations. This project will employ immunochemical techniques to study spatial and temporal relationships of surface antigen expression during hepatocarcinogenesis. Conventional antisera and monoclonal antibodies will be used to characterize the cell-surface antigens of primary and transplantable hepatocellular carcinomas of ACI rats. The antigenic profiles of the tumor cells will be analyzed by two-dimensional electrophoresis of immunoprecipitates. The profiles of tumors will be compared with those of normal and regenerating hepatocytes to detect changes in expression of organ-specific antigens. Specific antisera will be used to assess the contribution of fetal antigens and viral antigens to the fetal antigenic mosaic. Histocompatibility antigens will be subjected to extensive immunochemical analysis to detect alterations. Immunofluorescence microscopy and cleavable bivalent cross-linking reagents will be used to examine topographic relationships of histocompatibility and tumor-associated antigens on the cell surface. Immunohistological methods will be used to examine livers of rats undergoing hepatocarcinogenesis for presence of tumor-associated antigens, and the serum and livers of rats being fed hepatocarcinogens will be examined for presence of antigenic markers. These studies should provide insight into the role of surface structures in positional control and growth regulation and should contribute to our understanding of the relevance of altered self-recognition to tumor immunity.

353. PATHOLOGY OF THE DIGESTIVE TRACT MUCOUS MEMBRANE

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The summary for this project is under entry no. 10.

354. DETECTION OF EARLY PANCREATIC CANCER - ROLE OF RIBONUCLEASE, PANCREATIC ONCOFETAL ANTIGEN AND OTHER TUMOR-ASSOCIATED ANTIGENS (ABBREV)

Tytgat, G. N., Teunen, A., Huibregtse, K., de Vet, B., Hoek, F., Hoitsma, H. F., Universiteit van Amsterdam, Dept. of Medicine, *Spui 21, Amsterdam, Netherlands*

The summary for this project is under entry no. 30.

355. TUMOR ANTIGENS IN PANCREATIC CANCER

Chu, T. M., Douglass, H. O., Lee, C. L., Shimano, T., Loor, R., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

The objective of this proposal is to isolate and purify tumor antigens from pancreatic cancer. The tumor antigens are to be isolated from plasma membrane fraction of the pancreatic tumors, from the extracellular fluid (ascites fluid) and tumor tissues of pancreatic cancer patients. The antigens (free or in complexed form) will be purified by gel filtration, ion-exchange chromatography, affinity chromatography, immunoadsorbent technique, preparative polyacrylamide gel electrophoresis and isoelectric focusing. The chemical, physical and immunological characteristics of the antigens will be studied. Xenobiotic antisera will be raised with the purified antigens and specificity of the antiserum studied. If biochemical and immunologic data indicate that the antigens are specific for or associated with pancreatic cancer, immunodiagnostic procedures will be developed which can be of value in early detection of pancreatic cancer and in monitoring the efficacy of therapy currently available for pancreatic cancer patients. During the past two years of this grant period, a glycoprotein antigen, shown not to be a normal serum component, has been isolated from the ascites fluid of pancreatic cancer patients. A plasma membrane fraction has been prepared from pancreatic tumors which demonstrates a pancreas tumor-associated protease(s) activity. This plasma membrane fraction also contains antigen component(s) which has been used successfully in a leukocyte adherence inhibition assay specifically for diagnosis of pancreas cancer. Pancreatic tumors have been shown to contain perchloric acid soluble antigenic species different from those of colonic CEA. Further, IgM- and IgG-containing immune complexes have been identified in pancreatic cancer patients. The purpose of this renewal proposal is to continue our study of these pancreas cancer-specific or -associated tumor markers and their clinical application.

356. CHARACTERIZATION OF LAI-DEFINED PANCREAS TUMOR ANTIGENS

Howell, J. H., Goldrosen, M. H., Douglass, H. O., Roswell Park Memorial Inst., Dept. of Surgical Oncology, *666 Elm St., Buffalo, New York,* 14263, United States of America

Patients with pancreatic adenocarcinoma have an extremely high mortality rate. Curative surgery would be more common if early differential diagnosis were feasible. Our group has demonstrated that the micro-leukocyte adherence inhibition assay (micro-LAI) can successfully and specifically detect the presence of pancreatic cancer. In our continuing study to determine the sensitivity of the assay for detection of early cancer, we require a stable, standardized source of pancreas tumor antigen. This will be accomplished by propagating several established low-passage pancreatic adenocarcinoma cell lines in athymic (nude) mice. A membrane extract of each of the lines will be analyzed by micro-LAI and the most consistently reactive tumor line will be grown periodically in large quantity and stable soluble antigen will be prepared for routine use in the assay. Malignant effusion fluid and patients' sera will be biochemically fractionated and selected fractions will be analyzed for reactivity in the LAI assay. Xenogeneic antisera will be prepared against the purified antigen preparations and they will be immunochemically and biochemically compared by serological assays and by 2-dimensional isoelectric-focusing electrophoresis. Radio-iodinated, purified antigen will be prepared and a radioimmunoassay (RIA) will be developed using the most avid xenogeneic antitumor antibodies. Preliminary RIA screening of selected sera from our serum bank will be carried out in a blind fashion. A portion of the pancreas cancer specific antibodies will be labeled with iodine-131 and assayed to determine their suitability for use in external computerized photoscanning for tumor localization. This project will significantly improve the micro-LAI assay and the serological and biochemical data obtained will complement the clinical results.

357. PANCREATIC CARCINOMA IN CONTINUOUS CULTURE

Yunis, A. A., Schultz, D. R., Wu, M., Arimura, G. K., University of Miami, School of Medicine, Dept. of Medicine, *1400 N.W. 10th Ave., Box 520875, Coral Gables, Florida,* 33152, United States of America

Human pancreatic carcinoma in continuous culture is being used as an experimental model to investigate 3 areas in all of which considerable work has been done in this laboratory: 1. Purification of a tumor-related antigen is underway. This antigen has been identified and appears to be immunologically identical to oncofetal antigen. We plan to develop a radioimmunoassay for this antigen and proceed to do large-scale testing of human sera. The effect of the antibody to the antigen on the growth and tumorigenicity of MIA PaCa-2 in nude mice will also be tested. 2. We have identified two proteolytic activites in cultured MIA PaCa-2, a plasminogen activator and a membrane protease. The latter will be purified and its role in tumorigenicity and tumor invasiveness will be assessed. 3. Extensive screening of chemotherapeutic agents will be performed on the cultured cells. Drugs found to have some effect on growth will be examined in depth for their effects on the cell cycle and various metabolic aspects of the cells.

Particular attention is being given to the potential role of glutamine antagonists as anti-pancreatic cancer agents.

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G. ANTIGENS OF BRAIN AND OTHER NERVOUS TISSUE TUMORS

358. BRAIN ASSOCIATED HEMATOPOIETIC ANTIGENS

Golub, E. S., Purdue University, School of Science, Dept. of Biological Science, *Executive Bldg., West Lafayette, Indiana,* 47907, United States of America

The presence of brain associated hematopoietic antigen on cultured bone marrow cells (Dexter cultures) is being examined. Brain associated stem cell antigen and the brain associated antigen found on pre-T cells are being determined by treating the cultured cells with appropriately absorbed rabbit anti-mouse brain antiserum and testing their ability to generate CFU-S or Thy-1 positive cells in response to thymopoietin. Bone marrow cells are being cultured in the presence of thymic epithelium cells and the expression of brain associated theta antigen is being determined.

359. BRAIN-SPECIFIC ANTIGENS - GENETICS AND BIOCHEMISTRY

Bechtol, K. B., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania,* 19104, United States of America

This research is based on the hypothesis that cell-surface molecules play critical roles in the differentiation and regulation of complex systems such as the nervous system. To explore this hypothesis, differentiation and tumor-specific antigens of the mouse nervous system will be recognized and defined using monoclonal antibodies from hybridomas. Cell populations defined by their antigenic differences will be purified and used in genetic, biochemical and biological studies of the component cells of the nervous system and of their surface molecules.

360. CELL SURFACE ANTIGENS OF CHILDHOOD BRAIN TUMORS

Zelter, P. M., University of Texas, Health Science Center at San Antonio, Medical School, Dept. of Pediatrics, *7703 Floyd Curl Dr., San Antonio, Texas,* 78284, United States of America

OBJECTIVE: The overall objective of this project is to develop specific and sensitive serologic assays for brain tumor antigens which may provide improvements over techniques currently available for diagnosis, classification and monitoring of these neoplasms in children.

APPROACH: CSA (tumor related, fetal, differentiation type) of these brain tumors will be identified and later characterized, using serologic and molecular techniques which were developed in this laboratory. Next, three types of investigations using these CSA will be implemented: 1) determination of tumor CSA phenotype, 2) quantitation of soluble CSA in cerebrospinal fluid, serum, and/or urine by radioimmunoassay, and 3) determination of human anti-CSA antibody responses. The value of these tests for diagnosis, prognosis, and therapeutic assessment will be determined by correlation with clinical parameters.

361. TUMOR-SPECIFIC MEMBRANE ANTIGENS IN HUMAN GLIOMAS

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OBJECTIVE: Isolation and characterization of tumor-specific membrane antigens in human gliomas in order to establish biochemical and immunological assays for diagnosis of human brain tumors.

APPROACH: A tumor-specific antiserum raised against cell-membrane components is used as a tool for detection of tumor-specific membrane antigens in human gliomas. By the use of the nonionic detergent, Triton X-100 is solubilized and purified by preparative polyacrylamide electrophoresis. Antisera against these antigens are raised in rabbits and in sheep and absorbed to various components extracted from membranes of normal human brain and lymphocytes.

PROGRESS: By a rabbit antiserum against the Triton extract from membranes of human gliomas, 2 membrane associated proteins have been detected which were absent in normal brain tissue, normal glia cells grown in vitro and normal lymphocytes. By immunodiffusion, counter-current electrophoresis and immunofuorescence, these antigens have been detected in all the 25 gliomas tested so far.

Electrophoretic analysis of the immunoprecipitate revealed in addition to the two bands of the immunoglobulin, one band in the molecular weight region of 70,000 daltons and the other in the region of 12,000 daltons molecular weight. Twenty (85%) of 25 patients suffering from gliomas have precipitating antibodies in their sera, which cross-react with the Triton-extract from the membrane of human gliomas.

362. STUDY OF SURFACE ANTIGENIC CHARACTERISTICS OF HUMAN CULTURED GLIOBLASTOMA CELLS

de Tribolet, N., Zander, E., Schnegg, J. F., Carrel, S., University Hospital Center for the State of Vaud, Service de Neurochirurgie, *CH1011 Lausanne, Switzerland*

OBJECTIVE: To investigate surface antigenic properties of cultured human glioblastoma cells.

APPROACH: Membrane fractions of 2 established human glioblastoma cell lines are prepared by nitrogen cavitation. Antisera against each of the glioblastoma cell membrane preparations are raised in rabbits. The antisera are then extensively absorbed to remove species-specific and HLA activities. Subsequent absorptions are performed with unrelated tumor cells and finally with normal human brain. The specificity and cross-reactivity of the absorbed heteroantisera are tested with the complement-dependent and the antibody-dependent cell-mediated cytotoxicity assays. 51Cr-labelled cells from 8 different human glioblastoma cell lines are used as targets. The heteroantisera will be further characterized by immunoprecipitation analysis.

In a parallel study BALB/c mice are immunized with whole human glioblastoma cells, and monoclonal antibodies are produced by hybridomas.

PROGRESS: Using the monoclonal antibodies three types of antigens have been identified: two of them are present on human malignant gliomas exclusively, but the third one is present on neuroblastoma, melanoma and normal adult and fetal brain as well as on malignant glioma cells.

363. CELL SURFACE ANTIGENS OF GLIAL BRAIN TUMOR CELLS

Bigner, D. D., Lewis, C. W., Duke University, School of Medicine, Dept. of Pathology, *Box 3711, Durham, North Carolina*, 27710, United States of America

Surface antigens of human glial brain tumor cells and VM murine spontaneous anaplastic astrocytoma cells were investigated by binding (indirect membrane immunofluorescence) and complement-dependent cytotoxic antibody (14C-nicotinamide release and dye-exclusion) assays. Antisera from nonhuman primates immunized with glioblastoma multiforme tissue or cells from permanent human glial brain tumor cell lines have been extensively absorbed to analyze which human glioma cell surface antigens may be species-, tissue-, or glial tumor-specific; oncofetal; or cross-reactive with non-glial tumor cell antigens. We have established an inbred colony of VM/dk mice, an in vivo transplantable VM astrocytoma line, and 3 VM astrocytoma cell lines. We will extend the initial finding of human glial tumor-associated antigenicity with additional primate antisera now in production and an expanded panel of neoplastic and non-neoplastic adult and fetal glial and non-glial human cell lines. Syn-, allo-, and xeno-genetic antisera raised against the VM astrocytoma will be analyzed for activity against murine oncornaviruses; antisera suitably absorbed will be analyzed for astrocytoma-associated antibody activity. In both systems we will extract from culture supernatants or cell membrane preparations antigenically active moieties by monitoring the process in inhibition assays with absorbed specific antisera. Radioimmunoprecipitation of labeled reactive antigenic moieties will be followed by polyacrylamide gel electrophoresis. Partial purification of solubilized antigens will be performed by gel filtration, ion exchange chromatography, and other gentle methods of protein separation. Specific antisera and solubilized antigens will be used to determine if body fluids of patients or cancerous mice contain glioma-distinctive antigens, measurement of which may serve diagnostic, disease process-monitoring, or immunotherapeutic purposes.

BIBLIOGRAPHIC REFERENCES: Laerum, O.D., Bigner, D.D., Rajewsky, M.F., (eds.) Biology of Brain Tumors, International Union Against Cancer Technical Report Series - Volume 30, 209 pp. Geneva, 1978. Bigner, D.D.: Chapter IV, Role of Viruses in the Causation of Neural Neoplasia, pp. 85-99, in International Union Against Cancer Technical Report Series - Volume 30, 209 pp. Geneva, 1978.

364. EVALUATION OF HUMORAL RESPONSE OF GLIOMA PATIENTS TO POSSIBLE TUMOR-ASSOCIATED ANTIGENS USING ADDC ASSAY (ABBREV)

deTribolet, F. N., Zander, E. F., University Hospital Center for the State of Vaud, Service de Neurochirurgie, *CH1011 Lausanne, Switzerland*

OBJECTIVE: To study the humoral response of glioma patients to a possible common surface tumor antigen(s) using the antibody-dependent cell-mediated cytotoxicity (ADCC) assay.

APPROACH: Sera from patients with glial tumors were collected at several intervals in the course of the disease and tested in vitro using an ADCC assay. Cells from 8 long-term cultures of human glioblastoma were used as targets in a 6-hour 51Cr release assay using peripheral blood leukocytes from normal individuals as a source of effector cells. The same tests were repeated using rabbit complement.

PROGRESS: Sera from 59 patients were tested against cells from 8 glioblastoma lines. Ten patients were found to have cytotoxic antibodies against targets from one or more cell lines as compared to 5 out of 25 normal blood donors. Extensive cross-reactions between glioma cells were found. However, the cross-reactivity was incomplete and the pattern of reactivity different for each serum tested.

The positive sera were tested against 5 tumor cell lines of unrelated origin and found to have a similar cytotoxic activity as compared to that obtained with glioblastoma cells.

365. IMMUNOLOGICAL ANALYSIS OF THE SURFACE ANTIGENS OF HUMAN GLIAL BRAIN TUMOR CELLS

Wikstrand, C. J., Duke University, School of Medicine, Dept. of Pathology, *Box 3711, Durham, North Carolina*, 27710, United States of America

We have extensively characterized a large panel of established human tumor cell lines of gliomatous and non-central nervous system origin by the use of polyspecific heteroantisera raised in nonhuman primates. This analysis led to the demonstration of normal adult and fetal human brain-associated antigens, cross-reactive glioma antigens, and potential idiotypic glioma antigens on the cell membranes of cultured human glioma cells. In order to produce antibodies of greater specificity, we have developed the hybridoma monoclonal antibody technique and are producing anti-human glioma hybridoma antibodies. Several hybridomas have been rapidly screened by a cell surface radioimmunoassay and *14C-nicotinamide release assay*: reactivity patterns of various degrees of specificity have been detected, including hybridomas which react with all human cell cultures and those which react primarily with cells of neuroec-

todermal origin. In addition, we are analyzing the anti-glioma activity of human antisera resulting from the immunization of glioma patients with allogeneic glioma cells.

We propose to produce several anti-human glioma hybridomas by immunization with cultured glioma cells and tissue biopsy samples and to define the specificities detected as adult or fetal organ specific, cross-reactive neuroectodermal, glioma associated, or potentially idiotypic in origin, following the elimination of species-specific reactivity by adsorption with multiple lymphoid cell populations if necessary, and demonstration of the lack of HLA reactivity, readily induced by glioma cell immunization. We will then determine both cell surface and tissue distributions of the glioma-associated specificities in cell-surface radioimmunoassay, and 14C-nicotinamide release, indirect immunofluorescence, and peroxidase-labelling assays of dispersed cells and tissue sections with monoclonal antibodies and human antiglioma sera. The antibodies of defined specificity will then be used to monitor the isolation, preparation, and characterization of cell-free glioma-associated antigenic specificities.

366. IMMUNITY TO HUMAN CANCER - FUNCTIONAL COMPONENTS

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Studies are being conducted in three areas: 1) isolation of human glioma-associated cell surface antigens; 2) characterization of a substance, found in the serum of humans and mice that bear anaplastic tumors, that inhibits fundamental cell-cell interactions; 3) study of the capability of normal cells, unrelated to the immune system, which have the capacity to inhibit tumor cell replication and biological activity. These studies employ mixed aggregates of normal and neoplastic human cells.

BIBLIOGRAPHIC REFERENCES: Levy, N.L. Primary intracranial tumors. In: Immunodiagnosis of cancer (R.B. Herberman and K.R. McIntire, eds.) Marcel Dekker, New York, 1979, pp. 677-696. Wright, L.L., and Levy, N.L. Generation on infected fibroblasts of human T and non-T lymphocytes with specific cytotoxicity against measles virus-infected cells and whose activity was influenced by histocompatibility. J. Immunol 122:2379, 1979.

367. TUMOR AND DIFFERENTIATION ANTIGENS IN THE IMMUNE CONTROL OF NEUROBLASTOMA

Revoltella, R. P., Bertolini, L., Butler, R. H., Businaro, R., Bosman, C., Torre, A., Vigenti, E., Ghelardini, P., Brahe, C., Consiglio Nazional delle Ricerche, Lab. of Cell Biology, *Rome, Italy*

OBJECTIVE: To isolate and characterize structural antigens responsible for the specific anti-tumor immune response in neuroblastoma (NB) tumor-bearing A/J mice.

APPROACH: 1) Isolation of clones and somatic cell hybrids of NB which are high-, low-, or non-producing for specific alloantigenic structures; 2) isolation and immunochemical characterization using monoclonal antibodies of tissue-type and tumor-associated antigens from cloned NB cells; 3) protection of syngeneic mice against NB cell challenge by inoculation of individual NB-associated antigens.

PROGRESS: Various clones and hybrids of the C1300 NB cell line which differ in particular structures antigenic in a syngeneic system have been isolated. In addition, a new line of neuroblastoma, NB-SV, has recently been obtained by infecting embryonic sympathetic neurons from strain A mice with SV40 virus. Antigens have been solubilized from cloned NB cells and partially purified. These fractions retained antigenicity as defined by a variety of immunological tests, including radioimmunological methods using antibodies from syngeneic mice immunized against fixed NB cells or NB cell membranes. Monoclonal antibodies anti-NB have been obtained by somatic cell fusion between plasmacytoma cells, P3x63Ag8, and spleen lymphocytes from A/J mice a) NB tumor-bearing, b) immunized against NB cell membranes. The specificity of these antibodies is being investigated using purified C-type RNA-viral antigens and the solubilized fractions from N^{n} cells.

368. CHILDHOOD NEUROBLASTOMA - ANTIGENIC CHARACTERISTICS

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The prognosis of neuroblastoma, one of the most common childhood cancers, has not improved over the last two decades. It is almost universally fatal in older children with disseminated disease. In contrast, neuroblastoma of infants younger than one year may spontaneously remit, or it is frequently controlled and cured with current therapy. The long-term goal of this proposal is to critically define these factors and to determine their role in the clinical characteristics and therapeutic response of childhood neuroblastoma. Specifically we will determine if lymphocyte or serum mediated cytotoxicity against neuroblasts is due to normal differentiation antigens or to tumor specific antigens, compare the cytotoxicity of bone marrow, lymph node and blood lymphocytes against autologous or allogeneic neuroblasts, and establish if alpha-fetoprotein, detected on established neuroblastoma cell lines, is also expressed on viable neuroblasts from patients and investigate the effect of neuroblast-associated alpha-fetoprotein on host immune response. Systematic studies of these parameters should establish the biological differences between neuroblastoma of younger and older children and provide the knowledge required for the development of effective therapy.

369. IMMUNOCHEMICAL CHARACTERIZATION OF NEURONAL CELL SURFACE ANTIGENS

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The nerve cell surface presumably plays a critical role in nerve function and in nerve cell-cell recognition and may therefore reflect the pathways of differentiation of distinct nerve cell types. Schachner and co-workers have prepared a series of distinct antisera that specifically detect antigens unique to the cell surface of murine nervous tissue. I propose to biochemically characterize and isolate antigens detected by two of these antisera, designated NS-1 and NS-3. Tissue cultured cell lines bearing NS-1 or NS-3 antigens will be radiolabeled by cell surface labeling techniques specific for protein or carbohydrate and NS-1 or NS-3 antigens will be isolated from detergent solubilized cell fractions using NS-1 or NS-3 antisera. Antigens isolated by electrophoresis or thin layer chromatography will be used to generate more specific antisera that will facilitate biochemical identification and immunological fractionation of differentiated nerve cell types. These more specific antisera will also be useful in analyzing nerve cell-cell interactions in living nerve cells.

370. SURFACE ANTIGENS OF NEURAL CELLS

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Through the use of specific antisera we will study (1) the surface antigens found on cloned lines of cells from rat and mouse brain and (2) the expression of these same antigens in normal nervous tissue. These investigations will be aimed specifically at correlating cell surface markers with cell function and at mapping specific cell populations during the development of the mammalian nervous system.

The cloned cell lines used in these studies have been identified as neuronal or glial on the basis of electrophysiology and a series of ion flux assays which can detect receptors for neurotransmitters and voltage-dependent Na plus, K plus, and Ca plus plus channels. Six antigens (N1-N6) have been identified which are specific for cell lines classified as neuronal, and four others (G1-G4) have been shown to be specific for cell lines classified as glial. We will continue to enlarge our library of antigens by preparing new antisera against cell lines that are not well characterized by the existing antigens. We will also attempt to prepare more monospecific antisera by immunization with purified antigens and by making hybridomas to produce monoclonal antibodies.

Most of the antigens defined by these studies have been located in normal brain in a rough way by use of quantitative immunoabsorption. In addition, more detailed mapping of cells has been done in the case of G4, a high molecular weight glycoprotein which is present on a distinct population of cells in sections and primary cultures of cerebellum. Future studies will

emphasize mapping of various antigens as a function of development - i.e., the sequence of appearance of antigens, the origin and fate of cells bearing the antigens, and the types of cells expressing the antigens.

H. ANTIGENS OF BLADDER AND OTHER URINARY CANCERS

371. DETECTION AND ISOLATION OF HUMAN BLADDER ANTIGENS

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Human bladder tissue removed at surgery will be heterotransplanted subcutaneously into nude mice (NIH Swiss and BALB/c) and allowed to grow in that in vivo system for short periods. The human tumor cells will grow as solid tumors and retain both genetic and cell surface immunochemical characteristics which were present in the human host. Tumors will increase in mass 20-30-fold so that the isolation and identification of tumor-associated antigens from the tumor cell membranes can be accomplished. The extracted membrane proteins will be separated and chemically characterized by isoelectric focusing and polyacrylamide gel electrophoresis (PAGE). Immunological identification of separated proteins will be by migration of the acrylamide-separated proteins into agarose containing antihuman bladder sera and by radioimmunoassay (RIA) against heterologous antisera. Sera from patients with established bladder carcinoma will be tested for humoral responses to tumor-associated antigens and will be assessed both by cytotoxicity assays using short-term cultures of tumor cells removed from mice and by RIA analyses. Such studies should lead to the isolation of antigens which would thus be clinically useful in developing assays for early diagnosis of human bladder cancer. The system should provide an understanding of the relationships between tumor growth and immune response.

BIBLIOGRAPHIC REFERENCES: Sam D. Graham, Jr., Don D. Mickey and David F. Paulson. Detection of Metastatic Tumors in Nude Mice: Brief Communication. J.N.C.I. 60:715, 1978.

372. IDENTIFICATION OF ANTIGENS SPECIFIC TO BLADDER CANCER

O'Toole, C. M., University of Tennessee, Center for the Health Sciences, School of Medicine, Dept. of Urology, *62 S. Dunlap St., Memphis, Tennessee*, 38103, United States of America

The project is concerned with identification of the peripheral lymphoid cells with cytolytic activity towards autologous and allogeneic target cells of urothelial origin. Patients with transitional cell carcinoma of bladder (TCC) with localized disease have been shown to have effector cells capable of exerting elevated lysis of the allogeneic cell line T24 (derived from TCC). TCC patients with progressive disease do not exhibit this reactivity. A patient with chronic cystitis with heavy plasma cell infiltrates into the bladder mucosa has been identified who also has effector cells in the blood with selective cytolytic activity toward certain TCC cells in culture. This work is now being extended to search for cytolytic activity toward autologous urothelial cells in patients with TCC and cystitis. The effector cells will be characterized according to conventional surface markers as T, B or null cells.

373. SPECIFIC ANTIBODIES TO BLADDER CARCINOMA TUMOR ANTIGEN

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The objects of this proposal are to produce antibodies specific for human bladder carcinoma antigens, and to establish whether antibodies are present in these tumors.

The method or approach will be to use bladder cancers, fetal bladder, urine, and urine sediment as possible antigenic sources. Each bladder cancer used will be staged; tumors of Stage III and Stage IV will be used. To increase the yield of tumors and provide a reproducible starting material, additional amounts will be obtained by growing the tumors in athymic (nude) mice. Control antisera will be made from normal tissue. By using immunohistologic and soluble antigen-antibody techniques, and radioisotopically labeled tumor cell membranes, it is hoped that a specific antibody-antigen reaction can be demonstrated which is unique for some bladder tumors. If the specific antibody can be made, it will be tested against tumors of all stages, including Stage I and II.

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374. CHARACTERIZATION OF ORGAN-SPECIFIC DIFFERENTIATION ANTIGENS OF THE BLADDER

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Antisera have been raised to membrane and cytosol fractions of normal calf bladder epithelium (ICRF Scientific Report 1978 - report 158). After prolonged immunizations the antisera are cross-species reactive, and after appropriate absorbtions, can be rendered specific for urothelium. By immunofluorescence, maximal staining of normal mouse urothelium is seen in the terminally differentiated luminal cells, localizing predominantly in the asymmetric luminal membrane. The antigen is retained by cell lines established from human bladder carcinomas, and in in vitro transformed mouse-bladder cells. Preliminary analysis of immunoprecipitates by SDS-PAGE indicates that the expression of antigen varies in different cell lines. Work is in progress to affinity-purify the antibodies, with the eventual aim of a more detailed analysis of expression of antigen during transformation, and also in order to develop a radioassay for the estimation of antigen in clinical samples.

375. IMMUNOCYTOCHEMICAL LOCALIZATION OF UROTHELIAL ANTIGENS IN NORMAL MOUSE BLADDER & CELL LINES DERIVED AFTER TREATMENT WITH CHEMICAL CARCINOGENS

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Urothelial antigens were detected at the light and electron microscope levels using unfixed frozen and paraformaldehyde fixed sections and peroxidase-antiperoxidase - or indirect peroxidase - DAB techniques. The antiserum was raised in rabbits against calf bladder cytosol and absorbed against other epithelia (see Report 138). The antigen was localized in the urothelium of bladders from young adult and old mice and was also present in transformed cell lines derived from mouse bladder epithelium. At the electron microscope level, it was demonstrated that the majority of urothelial antigen was confined to the most differentiated superficial cell layer and was associated with structural cytoplasmic elements. In neoplastically transformed cells the localization was also cytoskeletal.

376. EXPERIMENTAL BLADDER TUMORS

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The rat model of transitional cell (TC) carcinomas of the urinary bladder has many similarities to the disease in the human. These tumors can be produced by a variety of stimuli (foreign bodies and carcinogens) and have been shown to have common cross-reacting antigens on in vitro measurements of cell mediated cytotoxicity as well as antigens unique to each tumor which can be demonstrated by transplantation immunity experiments.

We would like to investigate the relationship of the common and unique tumor antigens with antigens present in normal transitional epithelium of the bladder, and determine whether the nature of the carcinogenic stimulus makes a difference in the antigens expressed and whether these are neo-antigens. We plan to measure this by both in vivo transplantation experiments and in vitro CMI measurements.

We would like to extend our electron microscopy studies of the mouse bladder to study the EM characteristics (asymmet-

ric unit membrane, desmosomes and A-type virus particles) of these tumors in the rat in vivo and in tissue culture. If an A-type virus similar to that found in the mouse is found to be present in the rat, then evidence for viral antigens would be sought.

377. IMMUNOLOGIC RESPONSE IN PATIENTS WITH KIDNEY CARCINOMA TO KIDNEY CARCINOMA EXTRACTS

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OBJECTIVE: To isolate and purify tumor-associated antigens obtained from fresh specimens of kidney carcinoma.

APPROACH: Crude soluble material of renal carcinoma and normal renal tissue, obtained by 3 M KCl either directly from surgical specimens or from crude membrane preparations will be further purified by gel-filtration, ultracentrifugal flotation in KBr and polyacrylamide gel electrophoresis. The immunogenicity and specificity of these preparations will be assessed by delayed cutaneous hypersensitivity reaction (DCHR) and by two in vitro tests: the leukocyte adherence inhibition and the agarose leukocyte migration inhibition.

378. DETECTION OF UROGENITAL NORMAL AND NEOPLASTIC ANTIGENS

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A direct in vitro binding assay which is sensitive and quantitative for detection of specific antigens on the surface of cultured human urogenital tumor cells has been developed in our laboratory. The immunoglobulin (IgG) fractionated from rabbit antisera is radiolabeled with 125I to obtain sensitivity in measuring the antigens present on the test cells. Adsorption of IgG with normal non-urogenital tissue enables the detection of both organ specific antigens (OSA) and tumor associated antigens (TAA). Further adsorption with normal urogenital tissue allows the detection of TAA. This assay will be compared with two other sensitive and quantitative binding assays for detection of specific surface antigens. The most effective assay will be used to monitor the isolation of membrane associated OSA and TAA from human urogenital tumor tissues grown in athymic nude mice and from tissues obtained at surgery and autopsy. Purified antigens will be used for the preparation of specific antisera requiring minimal adsorption. The purified antigens will also be used to develop direct and blocking radioimmunoassays for detection of TAA and OSA in body fluids. Clinical testing will begin when quantitative adsorption experiments indicate that the adsorbed antibodies are specifically detecting OSA or TAA. Serum and urine from patients with different stages of urogenital malignant diseases will be tested for their ability to block the binding assay. The degree of blocking will indicate the amount of OSA and TAA in these body fluids. This information will be used to evaluate the binding assay as a method for early detection and monitoring of urogenital malignancies.

I. ANTIGENS OF PROSTATIC CANCER

379. ANTIGENIC COMPONENTS OF HUMAN PROSTATIC ADENOCARCINOMA

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Immunological approaches to human prostate cancer depend upon the existence of cell-surface antigens capable of evoking a specific immunological response. The purpose of our study is to determine whether patients with prostatic cancers develop an immunological response to antigens unique to their tumor cell surfaces. In addition to providing information about the fundamental cellular changes underlying malignant transformation, these studies have the immediate practical goals of providing the means to assess the immunological response of patients to their tumors during the course of disease both with and without treatment; of opening avenues for manipulating the immunological response in favor of the tumor-bearing host; and of developing immunological methods for the earlier and more accurate detection of prostatic cancers.

Carcinoma of the prostate provides an excellent model for studying cancer immunology in man because of its relatively high prevalence and chronic nature. It is a tumor whose progress can be slowed down by surgery and, in some cases, by hormonal therapy or chemotherapy. Because patients with prostatic carcinoma are in relatively good health during much of the course of their disease, they have an opportunity for developing an immune response to the tumors.

We plan to continue our studies in prostatic carcinoma by evaluating the specific immunologic response of patients to their own tumors. Cell-mediated as well as humoral responses will be assessed by several new approaches. These include in vivo and in vitro cytotoxicity assays as well as biochemical studies of prostatic tumor specific antigen. These investigations should provide a rational basis for immunotherapy aimed at modifying the response to this cancer in man.

BIBLIOGRAPHIC REFERENCES: B.-K. Choe, E.J. Pontes, I. McDonald and N.R. Rose. Purification and characterization of human prostatic acid phosphatase. Prep. Biochem. 8:73-89, 1978. B.-K. Choe, E.J. Pontes, N.R. Rose and M.D. Henderson. Expression of human prostatic acid phosphatase in a pancreatic islet cell carcinoma. Investigative Urology 15:312-318, 1978.

380. PURIFICATION OF PROSTATE CARCINOMA-ASSOCIATED ANTIGENS

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The lymphocyte hybridoma technique will be used to obtain monospecific antibodies against prostatic carcinoma-specific antigens employing the established prostatic carcinoma cell lines DU145 and PC3, cell suspensions from heterotransplants of these cell lines, or fresh prostate tumor tissue as immunogens. The production and specificity of the antibody secreting hybridomas will be monitored by a radioimmune cell-binding assay using a panel of cultured and fresh human normal and tumor cells. Clones secreting antibody specific to prostatic carcinoma cells will be maintained as ascites in BALB/c mice. The monospecific antibody will be used to prepare immunoabsorbents for purification of the prostatic carcinoma-specific antigen in 3M KCl extracts of prostatic tumor cells by biospecific affinity chromatography. Purification of the specific antigen will be monitored by gel electrophoresis, immunoelectrophoresis, and in vitro binding immunoassays. Physicochemical characterization and determination of whether the purified tumor specific antigen-cross reacts with prostatic acid phosphatase, oncofetal antigens, and HLA antigens will be performed using established procedures. The purified antigen and monospecific antibody will be used to develop a radioimmunoassay, and the potential value of this assay for diagnosis and monitoring of prostatic cancer patients before and after therapy will be assessed.

381. ANTIGEN MARKERS IN DIAGNOSIS OF PROSTATE CANCER

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The objective of this proposal is to continue our study on antigen markers which may be useful in the detection of prostate cancer. A new, specific and sensitive solid-phase immunoflourescence assay for acid phosphatase is to be evaluated for its potential value in the early diagnosis of prostate cancer. A prostate-specific antigen has been purified to homogeneity. The chemical, physical and immunological properties of this prostate antigen will be studied. Xenoantisera have been raised against the prostate antigen and the specificity of the antisera will be determined. Initial data indicate that this antigen may be of potential use in identifying prostate cancer patients. A sensitive immunoassay, such as radioimmunoassay or enzyme-immunoassay, will be developed and evaluated. In addition, a prostate tumor-specific antigen has been demonstrated and antiserum against it has been produced in non-human primates (baboons). Isolation, purification and characterization of this prostate cancer antigen will be performed. Furthermore, a prostate acid

phosphatase-binding globulin has been isolated from normal human serum by affinity chromatography using purified prostatic acid phosphatase. Its potential clinical application in prostate tumor radiolocalization and immunotherapy will be studied. Finally, an immunoassay for measurement of serum ribonuclease is being developed, as purification of two prostatic ribonucleases has been achieved.

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382. MEMBRANE ANTIGENS OF RAT PROSTATE ADENOCARCINOMA

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Two different sublines of the Dunning R3327 transplantable prostate adenocarcinoma are being investigated. These sublines, designated G and H at our institution, demonstrate in vivo and in vitro differences. The G subline is rapidly growing and poorly differentiated histologically, while the H subline grows very slowly and is a well-differentiated tumor.

Lectin binding studies have demonstrated quantitative differences between the two tumors. Purified membrane preparations from the H tumor bind more concanavalin A (Con A) and wheat germ agglutinins than do G tumor membrane preparations. These studies have been extended to monolayer cell cultures derived from G and H tumors. The H tumor cells bound more Con A than did the G cells, and further analysis of these kinds of experiments suggests a heterogeneity of binding sites of both G and H cells in culture.

Tumors and cells in culture analyzed by flow cytometry have demonstrated that the tumors consist of at least two populations of cells, one diploid and one aneuploid. Cell separation techniques and cloning experiments are underway to identify the tumor cell.

Antibody has been produced in goats to purified membranes of the G and H tumors and in rabbits to whole tumor cells. Fractionation of the immunoglobulins and affinity chromatography have resulted in the isolation of an antigen. The tumor specificity of this antigen is under investigation.

J. ANTIGENS OF OVARIAN AND GENITAL CANCERS, INCLUDING TERATOCARCINOMAS

383. ANTIGENS OF HUMAN OVARIAN TUMORS

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Ovarian cancer is second only to breast cancer as the most common cause of death from gynecological malignancy in women in this country. The principal objective of this project is to identify antigens or markers of ovarian tumors which can be used in an immunodiagnostic assay for the detection of early ovarian cancer. Xenogeneic sera will be used to identify (1) antigens in extracts of ovarian tumors and (2) cell surface and shed antigens of cultured ovarian tumor cell lines. Using rabbit antisera we have already identified two antigens (OvC-1 and OvC-2) present in ovarian tumors but not in normal ovaries. Both these antigens are present in amniotic fluid which is a convenient source of material for isolation of the antigens. OvC-1 is a new pregnancy-associated antigen. OvC-2 is a new oncofetal antigen and is found in a fetal intestine as well as in ovarian tumors. The development of sensitive assays (e.g. radioimmunoassays) capable of detecting these antigens in serum or plasma will enable us to determine their possible usefulness for the immunodiagnosis of ovarian cancer. Cultured ovarian tumor cells will be used in attempts to identify new antigens not previously identified when tumor extracts were used. Components shed from cultured ovarian tumor cells will also be studied. Antisera to the cultured cells and their released components will be raised in rabbits and monoclonal antibodies will be produced using the procedure of Kohler and Milstein. The specificity of the antisera will be studied by serological techniques using binding or adherence assays and by radioimmunoprecipitation. New antigens showing ovarian tumor specificity will be examined for their possible use in the early detection of ovarian cancer.

384. EVALUATION OF THE CLINICAL SIGNIFICANCE OF TUMOR MARKERS IN THE SERUM OF TESTIS TUMOR PATIENTS

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OBJECTIVE: To test the usefulness of a panel of tumor markers in order to diagnose as early as possible developing carcinomas of the testis, preferably before they become clinically manifest, and to evaluate the benefit of these markers in monitoring patients who are undergoing chemotherapy. 1) Evaluation of the clinical significance of markers known for other carcinoma. 2) Search for new tumor (testis) specific antigens.

APPROACH: The following potential markers will be included in the panel: AFP, CEA, HPL, beta-HCG, SP-1 and alpha 2 macroglobulin. Their values will be determined longitudinally before and after operation and in the serum of patients who are under control with or without chemotherapy. The other part of the approach will be devoted to extraction of tumor (testis) tissue in order to raise antisera for specific oncofetal antigens, as has been described for liver tissue (AFP), colon (CEA), lung tumors and malign melanoma.

PROGRESS: The results of a pilot-study concerning AFP and beta-HCG determinations confirm those reported by others. Both markers proved to be very reliable in detecting recurrence of carcinomas before these were clinically manifest. Chemotherapy could successfully be monitored regarding the AFP- and beta-HCG profiles.

385. DIAGNOSTIC APPLICATIONS OF HUMAN TUMOR ANTIGENS

Alexander, E. R., University of Washington, School of Public Health & Community Medicine, Dept. of Epidemiology, *4000 15th Ave. N.E., Seattle, Washington*, 98105, United States of America

OBJECTIVE: Develop and evaluate immunodiagnostic methods for recognizing epidermoid cervical cancer antigen and antibody by: purifying and characterizing the epidermoid cervical cancer antigen described in the proposal; developing quantitative radioimmunoassays for this antigen and related antibody; seeking methods for recognitions of this antigen in cells and/or tumors; studying distribution of the antigen or related antibody in the sera of selected populations of cancer patients, patients with cervicitis, and matched controls. When test appears to discriminate between cancer patients and controls, test a coded panel of serum specimens to be provided by NCI and determine the utility of a test in early diagnosis in an ongoing prospective study.

386. TERATOCARCINOMA AND EMBRYONAL TUMORS - SURFACE ANTIGENS

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The summary for this project is under entry no. 54.

387. ANTIGENS FROM GENITAL TUMORS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

388. VIRUS ASSOCIATED ANTIGENS IN GYNECOLOGIC CANCERS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

389. CELL SURFACE ANTIGENS OF TRANSFORMED CELLS - TERATOMAS

Levine, A. J., State University of New York, Health Science Center at Stony Brook, School of Basic Health Sciences, Dept. of Microbiology, *Stony Brook, New York*, 11790, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

K. ANTIGENS OF LUNG AND BREAST CANCER

390. ISOLATION OF SOLUBLE TUMOR-ASSOCIATED ANTIGENIC MARKERS FROM HUMAN TUMORS

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OBJECTIVE: To isolate, purify and characterize antigens associated with human tumors in order to develop clinically useful radioimmunoassays.

APPROACH: In addition to the work described in an earlier report submitted to the ICRDB the purification of lung tumor associated antigens is now carried out by using a radioimmune technique in soluble systems. Results obtained by this method are compared with those obtained by the initially used C'-dependent microcytotoxicity test. Preparations of different origin (pleural diffusions and surgical materials respectively) are being investigated.

PROGRESS: An account of the isolation and partial characterization of an antigen apparently associated with squamous cell carcinoma of the lung has been published (Wolf, Br. J. Cancer, 1978, 36, 1046). The material, a glycoprotein of approximately 1.7 times 10 to the 5th power MW (by gel filtration) has been isolated from pleural effusions of cancer patients, and experiments to assess its usefulness as a tracer in clinical trials are now in progress.

391. IMMUNOTHERAPY OF HUMAN CANCER

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The specific goals of this proposed research are as follows: 1. To define and characterize tumor-associated antigens of lung carcinoma. 2. To isolate and purify tumor-associated antigens and to use them to develop a radioimmunoassay for the detection of tumor-associated antigens in the sera and urine of lung cancer patients. 3. To evaluate in vitro assays of immune function to determine the usefulness of a particular immunologic assay and of a particular lung cancer antigen to accurately reflect total body burden of tumor.

Lung carcinoma antigens will be isolated in soluble form from abstracts of lung carcinoma tumor cells or spent medium of tissue culture cells. Antigens will be purified using fractional precipitation with ammonium sulfate, KBr flotation and column chromatograhy. Assays employed will be leukocyte migration inhibition and complement fixation for cellular immunity and isotope binding and immunofluorescence for serologic tests.

A group of lung carcinoma associated antigens has been identified. Antibodies directed against these antigens have been found in 50% of lung cancer patients tested. These antibodies have been shown to be not directed against HLA or other normal transplantation antigens, oncofetal antigens or tumor-associated antigens associated with some other histologic types of cancer.

This research program is highly relevant to the clinical situation. If new diagnostic and prognostic indicators of human lung carcinoma are discovered, the disease may be diagnosed in its early stages and chances of a cure for these patients will be markedly increased.

392. BIOCHEMICAL AND SEROLOGICAL STUDIES OF TUMOR-ASSOCIATED ANTIGENS AND OF LYMPHOKINES

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A radioimmunoassay (RIA) for a human lung tumor-associated antigen (hlTAA) is being evaluated for its potential use in diagnosis and/or monitoring of lung cancer patients. The antigen detected is immunochemically cross-reactive with a normal plasma protein, alpha 1-antichymotrypsin (ACT). Studies have been aimed at eliminating or circumventing interference caused by ACT in the RIA. We have demonstrated that the two proteins differ in their binding site for chymotrypsin since a) ACT totally inactivates the enzyme whereas the hlTAA has no effect on chymotrypsin activity, and b) only ACT will bind to insolubilized chymotrypsin/agarose. Initial attempts to remove all cross-reactive ACT from plasma using chymotrypsin/agarose have so far been unseccessful, but these studies will continue. The RIA in its present form has been used to examine sera from more that 130 normal individuals as well as from over 300 lung cancer patients. In addition 24 lung cancer patients have been sampled serially for periods of 5 to 19 months. In general there is positive correlation between marker level and disease status. A murine T cell hybridoma was prepared which, after cloning, is a stable producer of macrophage migration inhibitory factor and macrophage activating factor. It will be used as a source for future purification and characterization of these lymphokines.

BIBLIOGRAPHIC REFERENCES: Braatz, J.A., Redman, L.W., and Holden, H.T.: Biochemical studies on the fucose-binding ability of murine macrophage migration inhibition factor (MIF). In: de Weck, A.L., Kristensen, F., and Landy, M. (Eds.): Biochemical Characterization of Lymphokines. New York, Academic Press, 1980, pp. 87-91.

393. RADIOIMMUNOASSAY OF TUMORAL ANTIGENS IN LUNG CANCER

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OBJECTIVES: Clinical evaluation of radioimmunoassay (RIA) of a fraction of carcinoembryonic antigen (CEA); development of new techniques of RIA of other CEA fractions of higher purity; and identification of tumoral antigens of bronchopulmonary cancer and development of its RIA.

METHODS: The CEA fraction used for RIA was obtained by perchloric extraction and purified by chromatography in Sepharose 4B, Sephadex G 200 and by ionic interchange. The RIA technique was developed in our laboratory, with sera of 250 patients with lung cancer and cancer of the digestive tract analyzed to date. This technique has proven more successful in lung cancer. The new fraction which will be used in RIA was obtained from a later purification, performed on an earlier fraction by affinity and adsorption chromatography.

394. THE RELATIONSHIP OF THE IMMUNE RESPONSE TO TUMOR DEVELOPMENT AND THE PREPARATION OF XENOANTISERA TO TUMOR SPECIFIC ANTIGENS

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OBJECTIVES: To study immunoregulation of tumor immunity in a mouse tumor model (the P815 mastocytoma of DBA/2 mice). To purify tumor antigens from human tumors of various histological types.

APPROACH: An in vitro culture system for the generation of syngeneic cells cytotoxic for P815 cells has been developed and is being used for the study of antigen specific suppressor cells and their factors generated by tumor growth or exposure to tumor antigen.

Xenoantiserum, specific for apparently tumor unique components of human squamous cell carcinoma of the lung, has been prepared and used to monitor the purification of the tumor antigens.

PROGRESS: Suppressor cells and a soluble factor have been demonstrated in the thymuses and spleens of mice bearing P815 tumors which specifically suppress the in vitro generation of cells cytotoxic for P815. The cells can be killed by appropriate anti-Ia antiserum plus complement and the factor can be removed by adsorption with anti-Ia antiserum. Neither the cells nor the factor exhibits H-2 restriction when tested in radiation chimeras.

An antigen apparently unique to carcinoma of the lung has been isolated using the monitoring antiserum and standard bio-

chemical procedures. A similar approach is being used to purify an antigen from P815 mastocytoma.

395. MILK-DERIVED ANTIGENS IN BREAST CANCER

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Work has continued on the separation and purification of milk-derived antigens that can be detacted in histological sections of breast cancer.

In particular, attempts have been made to isolate one highly antigenic protein, antibodies to which have been found as contaminants in antisera prepared both by us and by other workers (ICRF Scientific Report 1978 - report 248).

Fresh human milk samples were processed to give the following fractions: a casein fraction; a proteose peptone fraction; a milk fat globule membrane fraction; and a whey protein fraction. Initial tests suggested that the proteose peptone fraction would be an ideal starting material but difficulties were experienced, probably due to denaturation during the preliminary separation.

A fraction containing the contaminant protein was prepared by anionic ion exchange chromatography and gel filtration chromatography. SDS polyacrylamide gel analysis showed it to be similar to the 'bound' protein eluted from an affinity chromatography column prepared with antibodies to the contaminant protein.

Experiments are also in hand to isolate the contaminant protein from the whey protein fraction by affinity chromatography, using a combination of immobilized lectins, and to investigate the possibility of the production of monoclonal antiboides to the contaminant protein.

396. PHYSICOCHEMICAL AND IMMUNOCHEMICAL ANALYSIS OF CANCER RELATED ANTIGENS

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OBJECTIVES: A. Exocrine ectopic secretion: breast cancer, as neoplasia of other origin, can secrete milk products such as casein and lactalbumin. Furthermore, a proteic tracer was identified in cystic fluid of mammary gland; this tracer is also detected in serum of patients with breast cancer. In the future, investigation will be directed to the immunological and physicochemical characterization of these mammary products. B. Isolation, purification and physicochemical and radioimmunochemical analyses of two new membrane antigens, one from bronchial neoplasm and the other from mammary carcinoma. C. Identification of antigenic groups of CEA which are organ cancer specific. D. Preparation and radioimmunoassays of viral antigens (P28, P47) involved in the mouse and monkey mammary carcinoma. Cross-reactions are presently detected by RIA, EIA, and immunofluorescence between these viral antigens and substances from cancer homogenate of patients with breast cancer. E. Isolation of immuncomplexes from blood of patients with breast and lung cancer. Characterization of the antigens involved in these immune complexes.

397. CHARACTERIZATION OF HUMAN BREAST CANCER GLYCOPROTEINS

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We have developed a unique model system for passive serum immunotherapy where immunological management of a highly lethal tumor, the ascites form of murine adenocarcinoma 755a, can be carried out reproducibly and with remarkable efficiency. Information concerning the mechanism of AD755a immunotherapy will be obtained through the isolation and characterization of the AD755a tumor-associated antigens and the serum factors responsible for providing protection. The type-C oncornavirus associated with the AD755a tumor will be isolated and the role of its structural polypeptides in the process will be determined.

The identity of AD755a tumor surface antigen(s) which induce and/or react with the protective serum will be analyzed by several analytical immunoassays, and attempts will be made to produce a protective serum using purified AD membrane antigens. The nature of the protective serum factor will be determined by isolating the class and subclass of antibody responsible for protection and the possible role of anti-idiotype antibodies in the serum will be analyzed.

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398. PURIFICATION OF BREAST TUMOR ASSOCIATED ANTIGENS

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OBJECTIVE: Isolate and purify the tumor associated antigen of the MCG-7 breast cancer derived tissue culture line for use in generating heteroantisera, to lead to the development of a radioimmunoassay for that antigen for use in immunological testing of patients with premalignant breast disease as well as patients with clinical carcinoma of the breast. Plans for monitoring of the antigen during purification and for analysis of clinical data shall be provided to the project officer for approval before such work is under way.

399. ANTI-Ii ANTIBODIES AND Ii ANTIGENS IN CARCINOMAS

Dube, V. E., Anderson, B. E., Khandekar, J. D., Northwestern University, Evanston Campus, Evanston Hospital, *2650 Ridge Ave., Evanston, Illinois*, 60201, United States of America

We have demonstrated increased concentrations of anti-I cold agglutinins in cancer sera, in particular in sera of patients with stage I-III breast carcinoma, and increased activities of Ii-antigens in sera of stage IV carcinoma patients. We plan to develop a RIA to quantitate anti-Ii activities and Ii-antigens in sera of individuals with various types of carcinoma, with an initial emphasis on breast carcinoma patients and on individuals with possible pre-malignant breast lesions. Ii-active glycoproteins and oligosaccharides will be used in inhibition assays of anti-Ii in cancer sera to assess whether certain immunochemical determinants are preferentially expressed. Ii-antigens from cancer sera and tissue will also be studied by their ability to inhibit immunochemically characterized anti-Ii sera. The results of these studies will help us understand the relationship of the expression of anti-Ii agglutinins and Ii antigens to the development and progression of carcinomas.

400. USE OF CELL MEMBRANES TO ASSESS PHENOTYPIC EXPRESSION

Ceriani, R. L., Peterson, J. A., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, *51st & Grove Sts., Oakland, California*, 94609, United States of America

The objective of this grant is to further define the normal mammary epithelial (ME) antigens, to study their altered expression in breast neoplasia and the reason for it, and finally to identify the effect of such alterations on ME cellular functions. Mammary epithelial (ME) antigens have been discovered by us both in humans (HME antigens) and in mouse (MME antigens). They are cell membrane components with cell-type specificity for normal mammary epithelial cells, which are also present in neoplastic ME cells. We will be using both models, human and mouse (complementing each other), to do the following: a) Continue to isolate and purify the ME antigens. This will be done using monoclonal antibodies of predefined specificity produced in hybrids between myeloma cells and spleen lymphocytes, the latter obtained from an animal immunized with mammary cell membrane components. Also, attempts will be made to separate ME antigens with the use of lectin affinity separation. b) Quantitate ME antigens with the aid of monoclonal antibodies in different stages and types of neoplasias, trying to correlate levels and possible changes with the neoplastic phenotype. The quantitation will be done by indirect immunofluorescence, radioimmunoassay, cytotoxicity and flow cytofluorometry. c) Study how ME antigen expression (presence, diminution, absence) affects function in ME cells, in such areas as cell

replication, differentiation and expression of the neoplastic phenotype.

Further, we will continue our maintained effort at cell identification in the breast by extending our study of specific myoepithelial antigen(s) and fibrocyte antigen(s) of the breast that we have just defined.

BIBLIOGRAPHIC REFERENCES: Ceriani, R.L., Peterson, J.A., Blank, E., and Miller, S.: Tumor Markers: Impact and Prospects. In: Applied Methods in Oncology, Vol. 2 (Boelsma, E. and Rumke, Ph., eds) Elsevier, Amsterdam, p. 101, 1979. Sebesteny, A., Taylor-Papadimitriou, J., Ceriani, R.L., Millis, R., Schmitt, C., and Trevan, D.: Primary human breast carcinomas transplantable in the nude mouse. J. Nat. Cancer Inst. 63:1331, 1979.

401. BIOCHEMISTRY OF MAMMARY TUMOR ANTIGENS

Tomana, M., Niedermeier, W., University of Alabama in Birmingham, School of Medicine, Dept. of Medicine, *1919 7th Ave. S., Birmingham, Alabama,* 35233, United States of America

The objective of this project is to isolate and characterize major mouse mammary tumor virus (MMTV)-related antigens from the plasma membranes of the mouse mammary tumor cells in order to elucidate the biochemical basis of the immunological cross-reactivity between the mouse mammary tumor and human breast carcinoma. The role of carbohydrates in the assembly, in biological properties of the mouse mammary tumor virion, and in MMTV-related antigens of the plasma membrane of mouse mammary tumor cells will be examined.

BALB/cf C3H mouse mammary cell line, grown in vivo and in vitro, is being used to produce MMTV, mouse mammary tumor cells and solid tumors. The antigens of MMTV isolated by ion exchange chromatography or affinity chromatography with plant lectins, gel filtration and/or preparative polyacrylamide gel electrophoresis in sodium dodecyl sulfate were used to prepare monospecific antisera in rabbits. Indirect immunofluorescence and radioimmunoassay were employed to determine the specificity of MMTV-related antigens in mouse and human tumors and the specificity of antibodies, in the sera of patients with breast carcinoma, that are responsible for immunological cross-reactivity with MMTV. Amino acid and carbohydrate analyses and determination of the molecular weight, pk of the protein and peptide maps will be carried out to characterize the MMTV-related antigens isolated from mouse and human tumors by affinity chromatography with immobilized antibody or physicochemical separation techniques.

BIBLIOGRAPHIC REFERENCES: Robidoux, S., Kajdos, A., Tomana, M., and Niedermeier, C.W.: Rejection of murine mammary tumours in BALB/c mice. Br. J. Cancer 39: 99, 1979. Tomana, M., Niedermeier, W., and Kajos, A.: Antibodies to MMTV-related antigen in sera of breast carcinoma patients. Protides of Biological Fluids 27:289-292. H. Peeters, ed. Pergamon Press: Oxford and New York, 1979.

402. CELL SURFACE MODULATION IN MAMMARY NEOPLASIA

Thompson, K., Abraham, S., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, *51st & Grove Sts., Oakland, California,* 94609, United States of America

Antigenic cell surface modulation associated with malignant transformation will be investigated in order to obtain a clear distinction between the normal, preneoplastic and neoplastic mammary epithelial cells. Initial studies on the cell surface components of normal and tumor mammary epithelial cells have established that the normal mammary cell surface membrane has an additional component which can be radiolabeled with either fucose or iodine. Further research will be aimed towards characterization of the antigenic structure of normal and tumor cell surfaces. Xenogeneic antibodies have been prepared to normal mammary epithelial cell-specific antigens and to virus-associated antigens. Syngeneic antibodies to mammary tumors (from tumor-bearing animals) have also been isolated. After appropriate absorptions, these antibodies will be used in immunofluorescence studies to determine the presence and location of the respective antigens and the interrelationship with other normal cell surface antigens (i.e., H-2, Thy. 1). In addition, these specific antibodies to normal and tumor-associated antigens will be utilized in radioimmunoprecipitation experiments followed by gel electrophoresis to characterize the respective antigens. Both the qualitative and quantitative differences in cell surface structure of normal and tumor mammary epithelial cells will be defined. Preneoplastic (HAN) epithelial cells will be compared to both normal and tumor mammary epithelial cells in order to assess early events in cell surface modulation accompanying the preneoplastic state.

403. MURINE MAMMARY TUMOR-SPECIFIC ANTIGENS (MICE, RABBITS)

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This is part of a broader project. A summary of this subproject is not available.

L. CIRCULATING ANTIGENS AND ANTIGENS OF OTHER SPECIFIC OR MULTIPLE CANCER SITES

404. DETECTION OF TUMOR SPECIFIC ANTIGENS IN THE CIRCULATION

Baldwin, R. W., University of Nottingham, *University Park, Nottingham NG7 2RD, England, United Kingdom*

Investigate whether or not circulating tumor antigens can be used as a diagnostic tool by following levels of circulating antigens during tumor growth in a variety of situations and attempting to relate the measured levels of antigen to tumor size. The experiments shall be carried out with a wide range of rat tumors being transplanted into syngeneic recipients. These tumors are 3-methylcholanthrene-induced sarcomas, hepatocellular carcinomas and spontaneously arising mammary adenocarcinomas in Wistar rats.

405. HUMAN TUMOR ANTIGENS - IMMUNE COMPLEXES AND CANCER

Gold, P., Shuster, J., McGill Cancer Research Centre, *3655 Drummond St., Montreal, Quebec,* H3G 1Y6, Canada

The tumor-bearing host develops a specific humoral antitumor immune response. Recently, new technology has demonstrated the presence of immune complexes in the circulation of cancer patients. The nature of these circulating complexes is unknown. Theoretically, these immune complexes should consist of tumor antigen and antitumor antibody. Our objective is to isolate such complexes, and analyze their individual components with the view to purifying the relevant tumor antigen. Paraformaldehyde fixed Raji-cells can be used to selectively bind and then elute the immune complexes present in cancer patients' sera. These complexes are presently being characterized.

To date, at least one constitutent has been demonstrated in the sera of cancer patients which may well have tumor antigenic activity.

406. ROLE OF CIRCULATING TUMOR ANTIGENS IN IMMUNOTHERAPY

Feldman, J. D., Scripps Clinic & Research Foundation, *476 Prospect St., La Jolla, California,* 92037, United States of America

OBJECTIVES: Isolation of TSA, VRA, and AgB. Measurement and metabolism of these three categories of antigens in the circulation in hosts with regressing and progressing tumors. Analysis of the immune response to each category of antigen, but especially to TSA; the analysis includes a dissection of the cellular response (macrophage activity, T and B lymphocyte participation) and of the humoral response (a quantitative comprehension of antibody response according to Ig class). The use of tumor antigens to accelerate and augment destruction of tumor and survival of host. Role of different antigen components in tumor progression (blocking, tolerance, etc.). Effect of antigen on specific and nonspecific responses of host.

407. ROLE OF CIRCULATING ANTIGEN IN TUMOR IMMUNITY

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The overall objective of this project is to study the role of tumor antigens in the immune response of the tumor-bearing

host to its tumor and to determine whether immunologic manipulations of the immune response of the host can increase the host's resistance against the tumor. There is ample evidence that tumors release antigen from the cell surface and that this antigen forms complexes with the existing antibody in the circulation. Although the respective roles of free antigen, antigen-antibody complex, or free antibody have been demonstrated in vitro, the role of these same materials in the host's immune response in vivo is less clear. Therefore, the specific objectives of the project are first, to isolate tumor antigen-antibody complexes from the tumor-bearing host and determine their effect on tumor growth in recipient animals, and second, to inject labeled tumor cells or tumor antigens into normal, immune, tumor-bearing or immunosuppressed syngeneic and allogeneic mice to determine the rate of clearance of these tumor cells and tumor antigens from the circulation. The effect of treatment of tumor-bearing hosts with cyclophosphamide or C. parvum on the rate of clearance of tumors will also be investigated. The ultimate goal of these experiments is to look for new and rational models of immunotherapy based on a better understanding of the effect of tumor growth on the immune response of the host.

BIBLIOGRAPHIC REFERENCES: Landes, R.G., Ristow, S.S. and McKhann, C.F.: A humoral suppressor factor in tumor bearing mice. Proceedings, American Association for Cancer Research 19:231, 1978.

408. ASSAY OF HUMAN TUMOR OR ORGAN ASSOCIATED ANTIGENS

Saravis, C. A., Zamcheck, N., Kupchik, H., Boston City Hospital, Mallory Gastrointestinal Research Laboratory, *818 Harrison Ave., Boston, Massachusetts*, 02118, United States of America

ZGM is a large tumor associated glycoprotein of demonstrable usefulness. Circulating levels of ZGM appear to reach a 'lid' of 150ng/ml, presumably because of its large size. We are now seeking a smaller molecule with ZGM activity. The purpose of this project is to isolate the circulating subunits of ZGM for incorporation in a ZGM radioimmunoassay for improved clinical use. ZGM will be isolated from the plasma of patients with adenocarcinomas and will be compared with ZGM isolated from tumor tissues using immunochemical and physicochemical procedures.

Tumor tissue ZGM will be degraded by use of hyaluronidase and neuraminidase, and treatment with sodium dodecyl sulfate, dithiothreitol, and trypsin. The subunits obtained will be radiolabeled, compared with radiolabeled plasma ZGM, and examined for differing inhibitory activity in the ZGM-RIA. We will select from plasma ZGM a ligand for measuring circulating ZGM more specific and sensitive than that isolated from tumor tissue. The clinical usefulness of the new ZGM-RIA will be tested in patients and compared with CEA-RIA analysis.

409. CIRCULATING TUMOR ANTIGEN TO EVALUATE RADIATION AND DRUG THERAPY (HAMSTERS)

Goldenberg, D. M., University of Kentucky, School of Medicine, Dept. of Radiation Medicine, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

This is part of a broader project. A summary of this subproject is not available.

410. STUDY OF THE NONSPECIFIC CROSS-REACTING ANTIGEN (NCA) AS A MARKER OF HUMAN MYELOID CELLS

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OBJECTIVE: To study the nonspecific cross-reacting antigen (NCA) as a marker of human myeloid cells.

APPROACH: Anti-NCA serum is used to stain cells of granulocytic and monocytic series in smears of peripheral white blood cells and bone marrow, as well as adherent monocytes prepared from peripheral blood, and colonies obtained by bone marrow culture. The same antiserum is used to assay NCA in the serum of patients with malignant blood diseases.

PROGRESS: NCA is a differentiation antigen of myeloid cells. In the granulocytic cell line, NCA appears initially in the promyelocyte stage. Its presence in leukemic myeloblasts and myelocytes is related to their differentiation. It is absent in leukemias with undifferentiated myeloblasts and is of no use in regard to the classification of these cases.

NCA is also found in monocytes-macrophages derived from blood or bone marrow, but not in leukemic monoblasts.

NCA is highly increased in the serum of patients with chronic myeloid leukemia, and decreased in cases of acute myeloid leukemia.

411. STUDIES ON IMMUNOGENICITY OF MEMBRANE SOLUBLE ANTIGENS EXTRACTED FROM NASOPHARYNGEAL CARCINOMA

Kawamura, A., Tsukuda, M., Sawaki, S., Takada, M., Yoshimura, N., Yanoma, S., University of Tokyo, Inst. of Medical Science, Dept. of Immunology, *Shirogunedai 4-6-1, Minato ku, Tokyo 108, Tokyo, Japan*

Nasopharyngeal carcinoma membrane soluble antigens (NPC MS-Ag) have been prepared from NPC-204 cultured cells by hypotonic saline membrane extract procedure (Hollinshead et al.). Qualitative and quantitative studies have been performed on specific immunogencity of these antigens.

From the standpoint of differences in EB virus specific and organ specific antigenicity, MS-Ag of other tumor cells, KB cells (EBNA negative NPC), Raji cells (Burkitt's lymphoma), and PC-1 cells (lung carcinoma) have been studied simultaneously.

In the future, following prospective studies, these NPC MS-Ag could be applied to estimate clinical status and specific immunotherapy of NPC patients.

412. ANTIGENIC DIFFERENCES BETWEEN HEMATOPOIETIC CELLS AND TUMOR CELLS (GROUNDWORK FOR SPECIFIC IMMUNOTHERAPY OF HUMAN CANCER)

Fitchen, J. H., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology/Oncology Section, *Wilshire & Sawtelle Blvds., Room 209, Los Angeles, California*, 90073, United States of America

The purpose of this study is to identify differences in cell surface antigens on normal bone marrow progenitor cells and cancer cells. Bone marrow progenitor cells are the cells that repopulate the bone marrow after bone marrow transplantation. Antibodies that detect antigens on subsets of normal blood cells, on leukemia cells, or on other types of cancer cells will be used to treat bone marrow samples to determine if the antibodies have damaged normal bone marrow progenitor cells. Particular attention will be given to antibodies that kill cancer cells but leave bone marrow progenitor cells unharmed. These antibodies will be employed in attempts to remove cancer cells from bone marrow samples known to be involved with cancer.

Knowledge gained from these experiments may allow the use of intensive chemotherapy in cancer patients with disease in the bone marrow, a group of patients in whom chemotherapy is often limited because of decreased tolerance to marrow-suppressive cancer drugs. The ability to remove cancer cells from diseased bone marrow will allow transplantation of that marrow back into the patient after intensive chemotherapy without the risk of giving back living cancer cells.

413. ISOLATION AND CHARACTERIZATION OF HETEROPHILE ANTIGENS

Merrick, J. M., State University of New York, Health Science Center at Buffalo, School of Medicine, Dept. of Microbiology, *3435 Main St., Buffalo, New York*, 14214, United States of America

This study will be primarily concerned with the isolation and characterization of the following heterophile antigens: infectious mononucleosis heterophile antigen (Paul-Bunnell antigen, PBA), Hanganutziu-Deicher antigens, and heterophile transplantation antigens. These antigens are detected on bovine erythrocyte membranes and can be extracted in soluble form. PBA activity is found in the minor glycoprotein fraction of bovine erythocyte membranes and has lipid-like properties. PBA will be further purified and its composition determined. Studies on amino acid sequence and oligosaccharide sequence and linkage will be carried out. PBA-like substances will also be studied on normal and malignant human lymphoid cells.

Hanganutziu-Deicher antigens (HDA) appear in the sera of patients with various malignancies, lymphoma, leukemia and other pathologic conditions. The high molecular weight glycoprotein fraction of bovine erythrocytes contains HDA that are related to the antigen found in human sera. This fraction, which

contains several other components, will be further fractionated. The structure of purified HDA will be examined and the antigenic determinants defined. The neutral glycolipid fractions of bovine erythrocyte membranes contain substances that are reactive with heterophile transplantation antibodies. The identity of these substances will be established and their structures determined.

BIBLIOGRAPHIC REFERENCES: Masaki, M., Nishimaki, J., Merrick, J.M., Fjelde, A., Kasukawa, R., Kano, K., and F. Milgrom. 1979. Paul-Bunnell Antigen in Pathologic Sera and Tissues. Fed. Proc. 38: 1224. Schroder, E.W., and Merrick, J.M., 1979. Alterations in Glycosphingolipid Patterns in a Line of African Green Monkey Kidney Cells Infected with Herpesvirus. J. Virol. 32: 734.

414. CHEMISTRY OF TUMORICIDAL MACROPHAGE SURFACE ANTIGENS

Springer, T. A., Kurzinger, K. W., Ho, M., Sullivan, C. P., Harvard University, Boston Campus, School of Medicine, Dept. of Pathology, *25 Shattuck St., Boston, Massachusetts,* 02115, United States of America

Mac-1, a granulocyte-monocyte specific differentiation antigen containing polypeptides of 190,000 and 105,000 MW and Mac-2 and Mac-3, monocyte specific antigens of 32,000 and 110,000 MW, respectively, have been identified using rat anti-mouse monoclonal antibodies. All these macrophage differentiation antigens are expressed in much greater quantities on activated macrophages than on monocytes in bone marrow, blood, or spleen. Mac-1 antigen is found on tumoricidal macrophages in the mouse and through cross-reaction on human monocytes and natural killer cells. Monoclonal antibody Fab fragments coupled to Sepharose will be used to isolate large quantities of radiolabeled antigens for chemical characterization. Subunit relationships will be studied by peptide mapping, cross-linking and gel filtration. The presence of functional and catalytic activities and intracellular phosphorylated sites will be investigated. Amino acid and carbohydrate compositions and NH2-terminals of proteolytic fragments and intact polypeptides isolated on a large scale will be determined. Arrangement in the membrane will be determined by digestion of whole cells, inside-out vesicles and purified detergent soluble antigen bound to Fab affinity beads and other procedures such as 32P, vectorial 125I, and photoactive hydrophobic reagent labelling.

415. NEUTROPHIL ANTIGENS - PATHOPHYSIOLOGY AND CHEMISTRY

Lalezari, P., Kumar, M., Montefiore Hospital & Medical Center, *111 E. 210th St., Bronx, New York,* 10467, United States of America

Maturation of human blood neutrophils is associated with development of specific antigens which have been shown to be present only on these cells. These antigens are controlled by several independent genetic loci, and their in vivo reactions with the corresponding antibodies have been shown to have important clinical implications. These include neonatal neutropenia due to fetal-maternal incompatibility, autoimmune neutropenia of infancy and adults, and pulmonary and most likely febrile transfusion reactions. It is the purpose of this investigation to broaden our present knowledge of the pathophysiology of neutrophil antigens, and then by using the neutrophil model, explore the possibility that similar organ-specific alloantigens with similar biological implications exist in other tissues. The study would include: 1. Search for new neutrophil-specific antigens, and procurement of new typing reagents by a) investigation of new cases of neonatal neutropenia, and b) production of xenogeneic antisera using purified antigens for immunization. 2. Development of new techniques for neutrophil antibody detection, including manual and instrumented passive hemagglutination tests, and quantitative complement fixation, antiglobulin consumption, and fluorescent microscopy. 3. Study of various immunological forms of neutropenia, and determination of their relation to neutrophil antigens. 4. Solubilization and chemical characterization of those antigens prepared by cell extraction, and found in urine and heavy density serum lipoproteins. 5. Determination of the importance of individual neutrophil antigens in blood transfusion reaction and bone marrow transplantation. 6. Demonstration of similar allospecificities in other organs, and evaluation of their implications in organ transplantation and autoimmune disease of the respective organs.

BIBLIOGRAPHIC REFERENCES: Lalezari, P., and Kumar, M.: Inhibition of cold insolubility of serum cryoglobulin by EACA. Am. J. Med. May, 1980, in press. Lalezari, P., and Pryce, S.C.: Detection of neutrophil and platelet antibodies in immunologically induced neutropenia and thrombocytopenia. In: Rose, N.R., and Friedman, H. eds., Manual of Clinical Immunology, second edition. 1980, in press.

416. MEMBRANE ANTIGEN ORGANIZATION IN TUMOR IMMUNITY

Gooding, L. R., Duke University, School of Medicine, Dept. of Microbiology & Immunology, *Box 3711, Durham, North Carolina,* 27710, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

V. OTHER STUDIES OF TUMOR ANTIGENS IN HUMANS

417. HUMAN TUMOR ANTIGENS - HUMAN TUMOR SPECIFIC IMMUNITY AND ISOLATION OF THE PUTATIVE TUMOR SPECIFIC ANTIGEN

Gold, P., Thomson, D. M., McGill Cancer Research Centre, *3655 Drummond St., Montreal, Quebec,* H3G 1Y6, Canada

The objectives of our research are twofold: (1) To study human tumor specific immunity with the computerized tube leukocyte adherence inhibition assay; (2) to use the host's tumor-specific immune response to monitor the isolation of the putative TSAs.

In the study of the host's tumor-specific immune response, the well-described computerized tube leukocyte adherence assay is used. Patients with cancer of the breast, lung, stomach, pancreas and colon will be tested and the control subjects will consist of patients with benign and premalignant diseases of a variety of organs. In the colon cancer study, the diagnostic capacity of the assay compared with other routine investigative procedures of barium enema and colonoscopy will be studied on outpatients with GIT symptoms. In the study of lung cancer, patients' blood samples are to be provided prior to diagnosis of treatment from the Montreal Chest Hospital to assess the reliability of the assay on samples provided by another centre.

The advantage of the computerized tube LAI assay is that it can be used to monitor isolation of the putative human TSAs. TSAs are being isolated from solid tumor, serum and urine of patients with colon and breast cancer as described previously.

Anti-beta-2 microglobulin affinity chromatography has been used to isolate TSAs water-solubilized by limited papain digestion from the membranes of tumor cells. The TSAs were shown to be associated with beta-2 microglobulin. IgG isolated from the serum of LAI positive patients is being used to isolate TSAs from the serum and urine of patients with metastatic colon or breast cancer. The presence of TSA activity is detected by the blocking tube LAI assay and the features of the material with and without TSA activity are being analyzed by two-dimensional gel electrophoresis. By this approach, the characteristics of human TSAs isolated from different tumors and sources may be defined.

418. HUMAN TUMOR-ASSOCIATED MOLECULAR EXPRESSIONS

Edgington, T. S., Scripps Clinic & Research Foundation, *476 Prospect St., La Jolla, California,* 92037, United States of America

These studies are devoted to immunobiology of human tumors. The experimental approach is twofold in nature, namely: 1) the identification and characterization of selected tumor-specific antigenic molecules which the tumor-bearing host may recognize and which may induce an immune response; 2) idenification of isolation and characterization of humoral and cellular immunoregulatory molecules of an antigen-independent nature which play a significant role in regulating the immune response of the host to tumors. This combined recognition, response and modulation of response appear to play a pivotal role in the biology of cancer.

419. NUCLEOLAR ANTIGENS OF HUMAN CANCER CELLS

Busch, H., Baylor College of Medicine, School of Medicine, Dept. of Pharmacology, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

The objectives of these studies are (1) to purify and characterize a nucleolar antigen which has been found in a broad range of human malignant tumors, (2) investigate the function of the antigen by initially localizing it in the nucleolus and assessing its effects on a variety of nucleolar enzymes and other components as well as its origin, and (3) to evaluate the potential immunodiagnostic use of the antigen by a number of techniques.

The specific aims of these studies are (1) the separation, isolation and purification of these antigens and preparation of the corresponding monospecific antibodies; (2) inasmuch as preliminary studies have indicated that the antigen(s) is localized to perinucleolar chromatin and intranucleolar chromatin in association with the fibrils and beads of the 'nucleolonemas', one goal of these studies is to determine what function the antigen has in these special locations; (3) to investigate the antigen in large series of specific tumors and determine relationships of growth rates and amounts of antigen present; and (4) to determine whether the antigen is related to growth, and whether it is a fetal gene product or a product of exogenous genes.

420. IN VITRO IMMUNIZATION WITH AUTOCHTHONOUS HUMAN TUMOR

Klein, E., Karolinska Institutet, *Solnavagen 1, S10401 Stockholm 60, Sweden*

OBJECTIVE: Demonstrate the existence of specific human tumor-associated antigens and show in vitro increased immune responses to such antigens after in vitro immunization with autochthonous tumor. Approximately 60 tumor specimens per year from patients with carcinoma of the lung, astrocytomas, and osteo- and soft tissue sarcomas will be studied. Attempts will be made to characterize the nature of the effector cell. Any modification of the study design must have the prior approval of the Project Officer before being instituted.

421. MONOCLONAL ANTIBODIES TO HUMAN TUMOR ANTIGENS

Takei, F., Lennox, E. S., Bleehen, N. M., Medical Research Council, Lab. of Molecular Biology, *15 Chaucer Rd., Cambridge CB2 2BF, England, United Kingdom*

OBJECTIVE: To detect and identify antigens which are expressed on human tumor cell surface but not in normal tissues in significant amount.

APPROACH: Monoclonal antibodies are raised against cultured human tumor cells. For this, mice and rats are immunized with the tumor cells (colon and lung carcinomas) and immune spleen cells fused with myeloma cells. Monoclonal antibodies raised in this way are screened on various tumor cells from tissue culture, normal tissues and sera. Antibodies which react with tumor cells but not normal tissues are selected and the antigens characterized.

PROGRESS: A number of monoclonal antibodies to colon carcinomas have been raised and are being characterized.

422. HUMAN CANCER SEROLOGY

Old, L. J., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

The two objectives of the Human Cancer Serology Program are: I. Serological definition and biochemical characterization of distinctive cell surface antigens of human cancer and II. immunovirologic analysis and biochemical characterization of human cancers of suspected viral etiology. The proposed studies relate to two central questions concerning human cancer: Do human tumor-specific antigens exist and do viruses play a role in the etiology of human cancer? The technique of autologous typing of malignant cells will be used to analyze cell surface antigens of malignant melanoma, renal cancer, astrocytoma, leukemia, lymphoma, and ovarian cancer, and will be applied to breast, lung, and colon cancer as improved methods for the in vitro propagation of these cancers are developed. Serological definition of tumor-specific cell surface antigens of human cancer will be followed by: a) Genetic analysis to determine the chromosomal loci specifying these antigens, b) search for natural antibody to these antigens in the normal human population, c) biochemical characterization, d) assessment of the clinical significance of humoral immunity to these antigens in patients with cancer, and e) development of maximally immunogenic forms of cancer vaccines. Immunovirological studies will focus on selected human cancers that are suspected from serological, virological, and epidemiological evidence to be related to herpesviruses or papovaviruses. The following associations will be investigated: The relation of cytomegalovirus to Kaposi's sarcoma, genital cancer, and colon cancer; the relation of Epstein-Barr virus to nasopharyngeal cancer, and the relation of BK and JC papovaviruses to brain cancer, renal cancer, and cancers developing in immunodeficient patients. Using a variety of serological assays and reference viral antigens and antibodies to identify the range of herpesvirus and papovavirus antigens, evidence will be sought for the presence of viral antigens in human cancer cells and for the occurrence of corresponding antibody in patients with cancer as compared with the normal population.

423. IMMUNOLOGICAL MARKERS APPLICABLE TO CYTOLOGY AUTOMATION

Aurelian, L., Johns Hopkins University, School of Medicine, Dept. of Microbiology, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

OBJECTIVE: Explore and characterize the antigens of normal, pre-malignant, and malignant human cells of types that are currently evaluated by manual cytologic techniques for the presence of pre-malignant and malignant changes. Qualitative and/or quantitative antigenic changes in pre-malignant and malignant cells which would allow discrimination from normal cells will be identified and characterized. The specificity and sensitivity of these changes will be evaluated with promising antigens. Assays should then be developed for application to human clinical cytology specimens.

424. CELLULAR ANTIGENS EXPRESSED IN TRANSFORMED CELLS AND TUMORS

Lo, A. H., State University of New York, Health Science Center at Stony Brook, School of Basic Health Sciences, Dept. of Microbiology, *Stony Brook, New York,* 11790, United States of America

Sera from animals bearing tumors initiated by an injection of viral transformed cells will be employed to immunoprecipitate tumor antigens from five murine and two human embryonal carcinoma cell lines. A search will be made for antigens common to tumor tissue, embryonal carcinoma cells and murine fetal tissue. The antigens detected in this screen will be characterized for fetal gene expression, partial peptide maps, expression in virus infected and transformed cells and the effect of virus mutants upon the expression of these proteins. Potential interactions between cellular and viral tumor antigens will be explored with the goal of establishing a function for these proteins. Ultimately, the regulation of these proteins in mice carrying developmental mutations will be studied at different stages of development of the fetus.

425. IMMUNE RESPONSE TO TUMOR CELL SURFACE ANTIGENS

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The objective of this project is to study both the induction and the effector step of cell-mediated cytotoxicity with the ultimate goal of finding ways and means to manipulate this effector arm of the immune system. The specific aims of this project are 1) to elucidate how immunoadjuvants like vitamin A derivatives stimulate cell-mediated cytotoxicity and how this observation can be used in the suppression of tumor growth in animal model systems, 2) to find out whether vitamin A derivatives have stimulating effects on the induction of cell-mediated cytotoxicity in an in vitro human model system, 3) to explore how the precursor cells of cytotoxic lymphocytes obtain their specificity for normal or modified histocompatibility antigens and whether the interaction of these lymphocytes with their targets requires receptors of one or several different specificities.

426. ANTIBODY RESPONSES OF TUMOR BEARERS TO THEIR TUMOR ASSOCIATED ANTIGENS

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No summary has been provided to the Smithsonian Science Information Exchange.

427. SOMATIC CELL GENETIC STUDY OF MAMMALIAN CELL SURFACE RECEPTORS AND ANTIGENS

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The objective of my current research is to dissect the genetic control of human cell surface functions with emphasis on the receptors for two antagonistic peptide hormones, insulin and glucagon. Attempts are being made to identify the genes responsible for production and regulation of the active receptor molecules and of the regulatory enzymes involved in the corresponding metabolic pathways; to understand the genetic variation in the receptor molecules and their effects on the functions of metabolic enzymes; to assign these genes to specific human chromosomes and localize them to specific regions of the chromosomes; and to understand coordination and/or hierarchy of gene action in the receptor-mediated hormonal signal transfer systems. The binding of a peptide hormone molecule to its specific receptor on the target cell surface is an important step in the regulation of mammalian cellular proliferation. Loss of such hormonal control might result in malignant cell formation.

I have been using somatic cell genetic approaches to achieve these goals in combination with biochemical and immunological techniques. A series of genetically altered mutant cells which are defective in each of the components essential for hormone action and for further specific steps in the signal transfer and in the metabolic sequences controlled will be isolated from a cell population mutagenized in vitro or from mice with identified diseases. Intra- and interspecific somatic cell hybrids will be produced using microcell-mediated chromosome transfer techniques in various combinations of mutant cells and wild-type cells of human and mouse origins.

My research is also directed toward the genetic dissection of human cell surface antigens, using specific antisera produced against highly segregated human x mouse somatic cell hybrids. New methods of assaying human phenotypes on a single cell basis will be developed with the use of specific antisera in combination with fluorescent and histochemical staining. Differences between normal and neoplastic cells in various aspects of cell membrane structure and function will be studied using genetically defined phenotypic markers.

The ultimate goal of my research project is to understand the mechanism of cellular response to external signals, using insulin and glucagon as model systems, and to obtain information on fine map of human genome and control of gene action.

428. IDIOTYPIC CIRCUITRY IN THE RESPONSE TO A MULTI-DETERMINANT PROTEIN ANTIGEN

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No summary has been provided to the Smithsonian Science Information Exchange.

VI. OTHER STUDIES OF TUMOR ANTIGENS IN ANIMALS

429. BIOLOGIC STUDIES OF SOLUBILIZED TUMOR ANTIGENS

McCoy, J. L., Litton Bionetics Inc., *5516 Nicholson Ln., Kensington, Maryland*, 20795, United States of America

The program is designed to provide a service testing laboratory to the National Cancer Institute to perform cell-mediated immunity assays capable of monitoring solubilized and purifed mouse tumor cell tumor-associated antigens and mouse H-2 histocompatibility antigens. A series of different tumor models including methylcholanthrene- and mineral oil-induced tumors as well as DNA and RNA virus-induced tumors will be employed. Litton Bionetics, Inc., (LBI) will use a colony of mice in which tumor transplants of the above neoplasms will be maintained. Further, animals will be actively immunized by a series of set protocols including use of X-irradiated cells, drug-induced tumor regression, or surgical removal of tumors with subsequent use of the lymphoid cells in Winn tumor-cell neutralization assays and in vitro cellular immunity tests with solubilized antigens.

430. TUMOR SPECIFIC ANTIGENS OF UV INDUCED TUMORS

Fortner, G. W., Kansas State University, School of Arts & Sciences, Dept. of Biology, *Anderson Hall, Manhattan, Kansas*, 66502, United States of America

OBJECTIVE: Test by in vitro and in vivo methodology for the presence of neoantigens on UVL-induced tumors to determine: if UVL-induced tumors have cross-reacting TSA as well as individually unique TSA; the presence or absence of these types of TSA on progressor tumors in relation to those found on regressor tumors; immune response and subsequent tumor progression.

APPROACH: The approach will be to immunize mice with regressor tumors and determine if this will protect them against progressor tumors of the same histological type. TSA on UVL-induced tumors will also be analyzed by indirect immunofluorescence with antisera prepared in rabbits and syngeneic mice against the progressing and regressing tumors. Adoptive transfer experiments with immune cells will be used to analyze suppression of the immune response by TSA.

431. IMMUNE MECHANISM OF RECOGNITION OF AND REACTION TOWARDS MURINE TUMORS

Forni, G., Cavallo, G., Giovarelli, M., Landolfo, S., Martinotti, M. G., Pugliese, A., Varesio, L., Universita degli Studi di Torino, Dept. of Microbiology, *Via Santena 7, 10126 Turin, Italy*

OBJECTIVE: The aim of this program is to analyze the relevance of membrane alloantigens in influencing the intensity and the characteristics of the immune response to various membrane antigens such as tumor transplantation antigens. This approach to the problem of the immunogenicity of neoplastic cells can be of great practical importance since in several cases experimental and human neoplasms display tumor associated antigens which are immunogenic in syngeneic hosts, but unable to trigger an efficient immunoreactivity. Therefore, a better understanding of the requirements for the elicitation of marked immune reactivities will allow a rational strategy for cancer immunotherapy.

APPROACH AND PROGRESS: a) Immunorecognition in vivo of BALB/c adenocarcinoma cells (ADK-1t) by H-2 matched DBA/2 hosts. We found that BALB/c B-lymphocytes and macrophages are the only cells able to trigger an effective immunoreaction to ADK-1t cells by DBA/2 hosts (Transplantation 27,435,79). Moreover, Ia alloantigens are required and the recognition is absolutely H-2 restricted (Immunogenetics 9,199,79). b) The spontaneous reactivity to ADK-1t and a Rous sarcoma virus induced tumor in A. SW mice is under a polygenic control. The genes involved map both in the H-2 complex and in the background. The effector cells responsible for a long-term spontaneous reactivity in vitro against these two tumors as well as the spontaneous reactivity towards protozoa (Trichomonas vaginalis) come from macrophage monocyte lineage (J. 'mmunol., in press). c) In vitro there is a discrepancy between proliferative and non-proliferative activation of T-lymphocytes (Transplantation 27,290,79). H-2 alloantigens trigger MIF, II-IF release and proliferative response in parallel. By contrast, when mice differing at minor alloantigens were tested, II-IF and MIF release took place even without going through proliferation (Immunogenetics 9,245,79). Proliferation, MIF and II-IF release are strictly T-dependent activities. However, while T-lymphocytes are the effector cells for the first two functions, II-IF is released by macrophages, but only after a specific T-lymphocyte signal (Transplantation, in press).

432. IMMUNOLOGY OF NATURALLY ARISING RAT TUMORS

Baldwin, R. W., Embleton, M. J., Middle, J. G., University of Nottingham, Cancer Research Campaign Lab., *University Park, c/o City Hospital, Nottingham NG7 2RD, England, United Kingdom*

OBJECTIVE: To determine whether naturally arising (spontaneous) rat tumors express tumor associated antigens recognized in the autochthonous host or syngeneic recipients.

APPROACH: The immunology of spontaneous tumors arising in WAB/Not rats has been studied at Nottingham since 1964.

This program has been extended to provide some 41 spontaneous tumors and these are now being studied. a) Tumor transplantation antigens are being detected by immunization and challenge experiments with tumors transplanted into syngeneic WAB/Not rats. b) Tumor cell surface antigens are being determined by antibody binding assays using antibody prepared in syngeneic rats. c) Cell-mediated immunity is being tested in vivo by the adoptive transfer of sensitized lymphoid cells. In vitro tests using short-term and long-term assays are being used to detect T lymphocyte and natural killer cell mediated reactions.

PROGRESS: Transplantation studies have demonstrated that unlike tumors induced with extrinsic agents, naturally arising tumors are at best only weakly immunogenic and some 2/3 of all the tumors tested did not induce significant levels of immunity. A similar pattern of reactivity was observed when tumor antigens were typed using antibody binding tests. Compatible with these findings was the observation that only 1/4 of mammary carcinomas were susceptible to active specific immunotherapy and most sarcomas were unsusceptible. The pattern of reactivity to lymphocyte sub-populations is being investigated and many are susceptible to natural killer cells.

433. CELLULAR MECHANISM OF IMMUNOLOGIC TOLERANCE AND CANCER ANTIGEN

Borel, Y., Childrens Hospital Medical Center, Dept. of Medicine, *300 Longwood Ave., Boston, Massachusetts,* 02115, United States of America

We have shown that antigen binding cells (ABC) are not eliminated in tolerance, and that the tolerogen (hapten-isologous IgG) persists on the surface of the ABC while the immunogen (hapten-heterologous protein) does not. Receptor blockade of the hapten binding cell is reversible both in vitro and in vivo.

This renewal proposal seeks to provide new information on the cellular basis of tolerance in an experimental model closely related to natural tolerance, using both functional and morphological techniques. The first objective is: a) To determine whether the ABC with tolerogen on its surface ('the tolerant cell') mediates tolerance; and b) to isolate the tolerant cell using immunoabsorbent techniques and cell sorter analysis. Secondly, using horseradish peroxidase, we intend to extend the principal of carrier induced tolerance to a conventional protein antigen. We will follow this readily identifiable enzyme marker as either a tolerogen or an immunogen on the surface as well as inside lymphoid cells by light and electron microscopy. Thirdly, the principle of carrier determined tolerance will be applied to an oncogenic viral antigen, gp70; we will determine whether gp70 conjugated to mouse IgG is tolerogenic in 3 ways: a) suppression of antibody to gp70; b) facilitation of the growth of leukemia; and c) improvement of murine lupus nephritis.

434. ANTIBODY RESPONSES OF TUMOR BEARERS TO THEIR TUMOR ASSOCIATED ANTIGENS

Klein, P. A., Scornik, J. C., Maino, V. C., State University System of Florida, University of Florida, School of Medicine, Dept. of Pathology, *1600 Archer Rd., Box J-266, Gainesville, Florida,* 32610, United States of America

Tumor-associated antigens (TAA) on the surface of tumor cells growing in vivo may induce cellular and antibody-mediated immunological reactions in the tumor-bearing host. These reactions may inhibit or enhance tumor growth at local sites or be involved in facilitating or preventing spread, implantation and survival of tumor metastasis. The major objective of this proposal is to analyze the antibody responses of tumor-bearing mice to TAA on their own growing tumors by 'capturing' and perptuating part of the host's anti-TAA antibody repertoire in the form of hybridomas secreting monospecific anti-TAA antibodies. These monospecific antibodies will be used to identify and study TAA on a series of recently derived immunogenetically well characterized chemically induced murine tumors. Known TAA on these tumor lines include tumor specific transplantation antigens (TSTA), histocompatibility antigens (H-2 and non-H-2) and murine leukemia virus-associated antigens. The study includes the following additional objectives: 1. To compare the anti-TAA antibody responses of the tumor bearing host to syngeneic tumors classified as 'immunogenic' or 'non-immunogenic' based on TSTA strength. 2. To determine, using specific monoclonal antibodies, the antigenic relationship of tumor cells found in metastatic foci to those cells present in the original parent tumor. 3. To examine the antibody response of mice to their primary methylcholanthrene-induced tumors before the tumors are transplanted for the first time. 4. To assess in a preliminary way several monoclonal anti-TAA antibodies as mediators of anti-tumor immune reactions, particularly in antibody dependent cellular cytotoxicity reactions.

435. STUDIES ON TUMOR CELL IMMUNOLOGY

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Cancers produced or promoted by environmental chemicals, radiation, or viruses have all been shown to display unique molecules on their cell surfaces (tumor specific antigens). These antigens are the targets of an individual's immune response against tumor cells. In addition, tumor specific antigens can serve as tags which differentiate tumor cells from normal cells. This project utilizes antibody molecules which bind to tumor specific antigens to target cytotoxic agents directly to tumor cells. Three areas of study are necessary: (1) Molecular identification of tumor specific antigens; (2) production of antibody to these antigens; and (3) coupling and delivery of drugs and other cytotoxic agents to the tumors. We have made antibody preparations to well define tumor specific antigens of virus induced leukemias and sarcomas of mice, and are studying the coupling and delivery of drugs to these tumors in vitro and in vivo. Two different lung tumors of mice (one spontaneous and one induced by methylcholanthrene) are being studied for tumor specific antigens. Antiserum to whole tumor cells is under study. Antisera to human leukemia/lymphoma cells are being used to detect specific molecules on the tumor cells. Other methods of targeting drugs to tumor cells are being explored.

436. ANTIBODY RESPONSES OF TUMOR BEARERS TO THEIR TUMORS

Klein, P. A., Scornik, J. C., Maino, V. C., State University System of Florida, University of Florida, School of Medicine, Dept. of Pathology, *1600 Archer Rd., Box J-266, Gainesville, Florida,* 32610, United States of America

Tumor-associated antigens (TAA) on the surface of tumor cells growing in vivo may induce cellular and antibody-mediated immunological reactions in the tumor-bearing host. These reactions may inhibit or enhance tumor growth at local sites or be involved in facilitating or preventing spread, implantation and survival of tumor metastasis. The major objective of this proposal is to analyze the antibody responses of tumor-bearing mice to TAA on their own growing tumors by 'capturing' and perpetuating part of the host's anti-TAA antibody repertoire in the form of hybridomas secreting monospecific anti-TAA antibodies. These monospecific antibodies will be used to identify and study TAA on a series of recently derived immunogenetically well characterized chemically induced murine tumors. Known TAA on these tumor lines include tumor specific transplantation antigens (TSTA), histocompatibility antigens H-2 and non-H-2) and murine leukemia virus-associated antigens. The study includes the following additional objectives: 1. To compare the anti-TAA antibody responses of the tumor bearing host to syngeneic tumors classified as 'immunogenic' or 'non-immunogenic' based on TSTA strength. 2. To determine, using specific monoclonal antibodies, the antigenic relationship of tumor cells found in metastatic foci to those cells present in the original parent tumor. 3. To examine the antibody response of mice to their primary methylcholanthrene-induced tumors before the tumors are transplanted for the first time. 4. To assess in a preliminary way several monoclonal anti-TAA antibodies as mediators of anti-tumor immune reactions, particularly in antibody dependent cellular cytotoxicity reactions.

437. IMMUNE RESPONSE TO MURINE TRANSPLANTATION AND TUMOR ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

VII. ANTIGENS OF HISTOCOMPATIBILITY COMPLEXES

A. HLA AND H-2 ANTIGENS

438. HLA-DR-LIKE ANTIGENS ON HUMAN MILK FAT GLOBULE MEMBRANE AND BREAST EPITHELIUM

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The milk fat globule membrane (MFGM) originates from the mammary epithelium during lactation by enveloping globules of fat during the secretory process. Suspensions of MFGs were positive when tested with a rabbit anti-DR serum or mouse hybridoma monoclonal anti-DR antibodies (DA2).

Examination of formalin-fixed preparations of breast tissue showed DR antigens to be localized on the secretory epithelium, although these were found only on lactating breast sections. Non-lactating breast, carcinomas or benign tumors, cultures of normal breast epithelium (R.C. Hallowes), a pleural effusion line MCF-7 or epithelial cells from milk (S. Chang) were negative.

Antisera raised against MFGM after absorption with epithelial membrane antigen (EMA) possessed residual anti-DR activity as judged by their ability to bind to DR-positive lymphoid cells or to precipitate two bands of c. 34,000 and 23,000 daltons from radio-labelled lymphoid DR-positive cell lines.

Further investigations will establish whether DR antigens from hemopoietic origin differ from those of epithelial origin and it is hoped that a large scale purification of DR antigens from MFGM will also be possible.

439. IDENTIFICATION OF HLA-D/DR DETERMINANTS

Thorsby, E., Solheim, B. G., Albrechtsen, D., Moen, T., National Hospital of Norway, Tissue Typing Laboratory, *Oslo 1, Norway*

OBJECTIVE: To identify and further characterize the HLA-D/DR determinants, on normal cells and malignant cells.

APPROACH: The DR determinants are identified by serology and the D determinants by cellular methods (HCT, PLT). The antisera are mainly produced by planned immunization.

PROGRESS: Our own serological reagents have been shown to identify DR antigens, and we have also demonstrated that it is possible by serology to type for the D determinants. Their further tissue distribution on normal and malignant cells is under study.

440. CHARACTERIZATION OF THE PHOSPHORYLATION SITE OF THE HLA-DR ANTIGENS

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The overall aim of this research is to characterize the phosphorylation site of the HLA-DR antigens, the human histocompatibility antigens located at the surface of cells of the immune system. This investigation will examine the role of phosphorylation in the regulation of movement of the DR antigens in the plasma membrane. The mechanism of regulation involves binding of the antigen to the cytoskeletal elements composed of actin. This movement has previously been shown to be associated with immune functions involving recognition and induction of proliferation.

The proposed research involves chemical cleavage of the DR polypeptide and isolation of the phosphate containing fragments which will then be sequenced. The actin binding properties of the HLA DR will be studied using competetive binding studies with myosin, spectroscopic chemical labels, chemical crosslinking and ultracentrifugation. The structural features of the putative actin binding site will then be characterized and the mechanism of its regulation by phosphorylation will be determined.

441. HUMAN HLA-D ANTIGENS

Strominger, J. L., Hemler, M. E., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts,* 02115, United States of America

A gene product from the human histocompatibility locus, HLA-D, has recently been purified from a B lymphoblastoid cell line. Initial studies of the dimeric structure showed features closely analogous to those of the polymorphic murine and guinea pig 'Ia' antigens found on the surface of several types of immunocompetent cells. Furthermore, like the murine Ia antigens, this human HLA-D antigen appears to play a role(s) in the regulation of the immune response. The structural basis for theoretical regulatory functions is not known.

Using specific heteroantisera against three different purified HLA-D antigens (which should have some overlapping but different specificities), I propose to locate and isolate human HLA-D antigens from several different cell types (T cells, B cells, macrophages and leukemic cell lines). Then I will comparatively examine their size and subunit structures and thus further determine the degree of analogy to the mouse and guinea pig systems. Following immunoprecipitation, initial structural characterization will be obtained using SDS-gel electrophoresis. After partial purification by affinity chromatography, peptide mapping will help establish the degree of homology between various subunits. Eventually, purification of HLA-D antigens from cell types other than B cells will allow more detailed immunochemical and biochemical analysis and provide an answer to the important question of whether HLA-D antigens on different cell types are products of the same gene or of closely linked genes.

442. STRUCTURAL AND BIOSYNTHETIC STUDIES OF HLA DETERMINANTS

Strominger, J. L., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Biochemistry & Molecular Biology, *Cambridge Station, Cambridge, Massachusetts,* 02138, United States of America

The specific goals of this research are to isolate and characterize the chemical structure and biochemical role of the human histocompatibility antigens, those substances involved in the phenomenon of rejection of transplanted tissue. The research objectives are summarized as follows: 1) To purify several HLA antigens (HLA-A, -B, -C, and -D locus products) from the surface of cultured human lymphocyte cell lines, using both detergent and papain solubilization; 2) to carry out structural studies on purified HLA antigens in order to examine the question of the nature of the specific immunological determinant in each; 3) to sequence the heavy chain of several HLA antigens in order to determine the extent of sequence homology to human immunoglobulins; 4) to study the cleavage of the detergent-solubilized HLA antigens by papain and to study the nature of the fragments released by papain to determine how these molecules are inserted into the lymphocyte membrane; 5) to study the nature of homology among the products of the major histocompatibility complex in man (i.e., HLA-A, -B, -C, and -D loci antigens); 6) to study aspects of pathology relating to HLA antigens, notably the linkage between HLA-B27 and susceptibility to several autoimmune diseases, and the high linkage between the Singapore-2 allele and susceptibility to nasopharyngeal carcinoma; and 7) to study functions of HLA antigens, possibly in cell-cell interactions in the immune response and in differentiation.

BIBLIOGRAPHIC REFERENCES: Lancet, D., Parham, P. and Strominger, J.L. The heavy chain of HLA-A and HLA-B antigens is conformationally labile: a possible role for beta 2-microglobulin. Proc. Natl. Acad. Sci. USA 76, 3844-3848, 1979. Orr, H.T., Lopez de Castro, J.A., Parham, P., Ploegh, H.L. and Strominger, J.L. Comparison of amino acid sequences of two human histocompatibility antigens, HLA-A2 and HLA-B7: the location of putative alloantigenic sites. Proc. Natl. Acad. Sci. USA 76: 4395-4399, 1979.

443. STRUCTURAL DIVERSITY OF HUMAN DR ANTIGENS - A MOLECULAR MODEL FOR CELL INTERACTIONS

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The human DR antigens are cell surface proteins that mediate some of the cellular interactions required for the generation of immune responses. They display serologic and structural diversity which is reflected functionally by the inability of cells derived from individuals expressing non-identical DR antigens to interact. Thus, DR antigens provide a useful model for correlating molecular structure with biologic function.

DR antigens consist of two polypeptide chains, one relatively invariant (alpha) and the other variant (beta) in structure. The aim of this proposal is to compare the structural variation of beta chains of DR antigens isolated from several B lymphoid cell lines expressing different DR allotypes. We will employ methods previously used for characterizing membrane products of the murine major histocompatibility complex. These include amino acid sequence and peptide map analysis. This knowledge will ultimately provide the molecular basis for defining the function of DR antigens and may provide a molecular explanation for the association of specific DR antigens with susceptibility to certain diseases.

444. CHEMICAL STRUCTURE OF IMMUNOGLOBULIN MOLECULES, ALLOTYPES AND HUMAN HISTOCOMPATIBILITY ANTIGENS (ABBREV)

Appella, E., Inman, J., Rogers, M., DuBois, G. C., Robinson, E. A., DeLeo, A., Jay, G., Ferrini, U., Seidman, J., Perham, R., Ramanathan, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Biology Laboratory, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Chemical and Structural Studies of Antibodies, Histocompatibility Antigens, Tumor Antigens and Epoxide Hydrase: It is the purpose of this project to study the chemistry and structure of different molecules. The amino acid sequence of light and heavy chain immunoglobulins is obtained in order to gain some insight into the nature of antibody specificity and the generation of diversity. Biochemical isolation of histocompatibility antigens, tumor antigens and epoxide hydrase is carried out. The chemical structure of these proteins is being approached by micro methods and the relationship of the amino acid sequence to immunoglobulin structure or other known structures is being analyzed. Cloning of histocompatibility antigens is being approached in order to study the molecular organization of the system and permit a better understanding of the nature of the extensive polymorphism at the H-2 loci.

BIBLIOGRAPHIC REFERENCES: Law, L.W., Rogers, M.J., and Appella, E.: Tumor antigens on neoplasms induced by chemical carcinogens and by DNA- and RNA-containing viruses. Properties of the solubilized antigens. In Weinhouse, S., and Klein, G. (eds.): Advances in Cancer Research, New York, Academic Press, 1980, Vol. 32, pp. 201-235. Appella, E.: Amino acid sequence of the light chain variable region of M511, a phosphorylcholine-binding murine myeloma protein. Molecular Immunology, in press, 1980.

445. CHARACTERIZATION OF HLA ANTIGENS OF DONOR'S LYMPHOCYTES

Duquesnoy, R. J., Blood Center of Southeastern Wisconsin Inc., *1701 W. Wisconsin Ave., Box 10-G, Milwaukee, Wisconsin*, 53201, United States of America

The summary for this project is under entry no. 238.

446. HISTOCOMPATIBILITY COMPLEX AND THE EXPRESSION OF ANTIGEN SYSTEM IN LEUKEMIA

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The summary for this project is under entry no. 241.

447. BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF TRANSPLANTATION ANTIGENS

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The purpose of this work is to examine the chemical, biological and immunological properties of histocompatibility antigens (H-2) and tumor specific transplantation antigens (TSTA). The approach is to purify the molecules expressing these antigens and determine their properties at each stage of the purification. These properties include: 1) Ability to induce in vivo transplantation rejection and humoral response, 2) ability to stimulate lymphoid cells in vitro, 3) chemical properties with particular emphasis on how these molecules bind to biological membranes, 4) the metabolism of these antigens in normal and neoplastic cells. The rationale for this approach is that through purification of these antigens one can determine if there are any structural similarities which may explain their common biological activity, i.e., transplantation rejection. Furthermore, knowledge of the biological properties of these molecules will provide a rational basis for using them in in vivo therapeutic protocols. Finally, when they are purified, the chemical structure of these antigens will aid in the understanding of the organization of the genes which encode them.

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448. ANTIGENS DETERMINED BY THE MURINE MAJOR HISTOCOMPATIBILITY LOCUS

Sachs, D. H., Ozato, K., Epstein, S., Bluestone, J., Auchincloss, H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Transplantation Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Studies are being directed toward understanding the major histocompatibility complex, the structure and function of the products of this complex, and manipulations of immune responses to these products. Current studies include: 1) Characterization of major histocompatibility antigens: Congenic resistant strains of mice are developed, maintained, and used in serologic and immunochemical analyses of the MHC products of the mouse. 2) Studies of monoclonal antibodies to H-2 and Ia antigens: Hybridoma cell lines are produced by fusion of immune mouse spleen cells with mouse myeloma cells. The monoclonal anti-H-2 and anti-Ia antibodies produced by these hybridomas are analyzed by serologic and immunochemical means and are used to further characterize the fine structure of the MHC. 3) Characterization of receptor sites for histocompatibility antigens: Anti-idiotypic antisera are being produced against anti-H-2 and anti-Ia hybridoma antibodies, and the effects of these antisera on in vitro and in vivo parameters of histocompatibility are being assessed. 4) Mechanism of tolerance to H-2 and Ia antigens: The humoral and cellular responses of radiation bone marrow chimeras are being examined, and the mechanism for maintenance of tolerance in these animals is being studied.

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449. CHEMISTRY AND DEVELOPMENT OF TRANSPLANTATION ANTIGENS

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Cell surface membranes are to be studied with serological and physical techniques. Diffusion of antigens in the plane of the membrane will be examined by three different techniques. Modification of membrane lipids will be performed and the diffusion rates for H-2 antigens will be correlated with physical changes measured by ESR and fluorescence methods.

Antigen expression will be monitored in normal differentiating systems, and correlated with physical changes in membrane structure, such as changes in membrane viscosity. H-2 and tumor antigen expression will also be measured in deliberately modified cell membranes. Studies of the binding of labelled

hormones to cell surfaces, as well as on the isolation of hormone receptors, will be continued in an effort to determine the effect of genes linked to the major histocompatibility complex on cell responses to polypeptide hormones.

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450. MICROSEQUENCE STUDIES OF PROTEINS INVOLVED IN THE ACTIVITIES OF CELL SURFACES

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Two classes of molecules on the cell surface which can trigger the rejection of various kinds of grafts are the transplantation antigens and the tumor specific antigens. Preliminary amino acid sequence information on the H-2K and the H-2D transplantation antigens of the mouse and their human counterparts has left a number of key questions unanswered regarding origin, function, and evolutionary interrelationships of these molecules. Molecular precursors or successors of these molecules are being identified in order to determine whether the molecules possess obvious functional regions such as the variable and constant regions of the immunoglobulin molecules. The point at which the mouse transplantation antigens diverge from one another and from their human counterparts is under investigation. Studies are also being undertaken to learn if antigens from various tumors are members of a homogeneous class of molecules and if any or all of them are related to any known class of normal cell surface antigens.

451. STRUCTURE OF MURINE HISTOCOMPATIBILITY ALLOANTIGENS

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The research proposed in this application is to ascertain the amino acid sequence of the H-2Kk histocompatability alloantigen expressed on the surface of ASL1-W murine leukemic cells. The first 30 N-terminal amino acid residues of H-2Dd will also be defined so that comparisons of the H-2Kk and H-2Dd gene products to each other and to other membrane-associated components vital for cellular interactions between host-derived and malignant or transformed cell may be assessed. Ten to the 12th power cells per week will be produced utilizing large-scale mammalian cell culture to provide sufficient H-2Kk alloantigen for study. Isolation and structural characterization of the H-2 alloantigens will embody features of high pressure liquid chromatography and semi-micro solid-phase technology to permit rapid delineation of their amino acid sequence. These investigations will undoubtedly lead to a better understanding of the structural basis for receptor-recognition phenomenon involved in tissue compatibility, differentiation, organogenesis, malignant cell transformation, virus susceptibility, and induction of the immune response to antigens. The majority of the methods and techniques developed in this project will be directly applicable to primary structure determination of other membrane proteins known to be important in both cellular and humoral immune responses.

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452. STRUCTURES OF HISTOCOMPATIBILITY-2 MEMBRANE ANTIGENS

Sears, D. W., University of California, Santa Barbara Campus, School of Letters & Science, Dept. of Biological Sciences, *Santa Barbara, California*, 93106, United States of America

The objective of this proposal is to characterize the structures of H-2 antigens. This study will focus primarily on the conformations of the membrane-bound glycoproteins coded for by H-2k and H-2K regions of the major histocompatibility complex of the mouse.

The research plan will be directed in essentially four areas: 1) Several methods will be tested for improving the yields and separation of H-2b antigens as presently isolated in highly purified form from EL-4 cells by a purification scheme developed, in part, by the Principal Investigator. The aim here is to purify H-2b antigens from this lymphocytic leukemia-derived cell line for structural examination by conformationally sensitive spectroscopic probes, such as circular dichroism and fluorescence, and by amino acid composition and sequence analysis. 2) In an extension of current studies by the Principal Investigator, various molecules with public antigenic specificities of the H-2.28 family will be compared by tryptic peptide map analysis. The aim is to clarify the structural basis of this complex family of antigens which has recently led to the discovery of a new class of H-2D antigens. 3) The nature of the antigenic sites of H-2 antigens will be explored by chemical reagents which alter serological reactivity. Peptides protected from modification by specific alloantibody, and therefore presumably in or near antigenic sites, will be identified by tryptic peptide mapping techniques.

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453. IA ANTIGENIC SPECIFICITIES IN MOUSE AND MAN

McKenzie, I. F., University of Melbourne, *Grattan St., Parkville, Victoria 3052, Australia*

We propose to study the chemistry, genetics, immunology and physiology of Ia antigenic specificities secreted into serum in a murine model and in man. Studies by other investigators have already demonstrated the importance of these specificities in the MLR, in T-B cell co-operation and in graft rejection. Our studies have already demonstrated the presence of Ia substances in serum and that these can be detected by a rabbit antiserum as well as by the conventional alloantibodies. We have also shown that Ia substances of low molecular weight are secreted by helper T cells after contact with antigen, and that this material is predominantly carbohydrate. We have also made substantial progress with this model in man. We now intend to study in the murine Ia model: a) The carbohydrate chemistry of the Ia specificities: structure of high molecular weight and low molecular weight components and further chemical analysis using enzyme studies; b) to examine secretion in tumour bearing mice (in allogeneic and syngeneic recipients) and after treatment with carcinogenic agents; c) examine the secretion in rats undergoing renal allograft rejection; d) examine secretion in mice under conditions which enable determination of the specificity of the Ia secreted and its relations to antigenic stimulation, e) to determine, in a GVH model, the genetic control of secretion of I region specificities. We also intend to further our genetic studies of H (Ia) - the human equivalent to the Ia specificities and to exploit the demonstrated homologies between murine and human Ia to study the chemistry, immunology and physiology of Ia secretion in man. In particular we wish to determine whether the measurement of Ia levels is equivalent to monitoring T cell function. Thus far, it seems that it is, but two circumstances require intensive study. a) the rejection of human renal allografts, b) examination of the serum of patients with cancer.

BIBLIOGRAPHIC REFERENCES: Parish, C.R. and McKenzie, I.F.C.: A Detailed Serological Analysis of a Xenogeneic Anti-Ia Serum. Immunogenetics 6:183-196 (1978). Parish, C.R. and McKenzie, I.F.C.: Carbohydrate-defined Antigens Controlled by the I-Region. Current Trends in Histocompatibility, Plenum Publishers, New York, (in press) (1979).

454. CHARACTERIZATION OF NEW HUMAN IA AND LEUKEMIA ANTIGEN

Mohanakumar, T., Virginia Commonwealth University, Medical College of Virginia, Dept. of Surgery, *1200 E. Broad St., Richmond, Virginia*, 23298, United States of America

The summary for this project is under entry no. 240

455. IA-LIKE ANTIGENS AND OTHER MEMBRANE GLYCOPROTEINS IN HUMAN LEUKEMIA

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The summary for this project is under entry no. 216.

456. PRIMARY STRUCTURE OF MHC I REGION ASSOCIATED ANTIGENS

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Proteins encoded by the I region of the major histocompatibility region (Ia antigens) will be isolated from Ia-bearing tumor cells. The cells will be radiolabeled with 3H, 35S and/or 14C amino acids, detergent solubilized and isolated with immunoadsorbants, utilizing monoclonal anti-Ia alloantisera from hybridomas. Protocols for isolating cyanogen bromide and proteolytic fragments from both the alpha and beta chains of the Ia molecules labeled with a variety of 3H amino acids and 35S cys will be initially developed. These fragment isolation procedures will be designed to maximize the recoveries of the radiolabeled products. Once the peptide isolation procedures are established, Ia molecules will be uniformly radiolabeled with 14C, the peptides isolated and sequenced.

457. STRUCTURAL AND SEROLOGICAL STUDIES OF IA ANTIGENS

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The Ia alloantigens are the only isolated gene products of the immune response control region (I region) of the mouse major histocompatibility complex (MHC). The molecules bearing Ia alloantigenic determinants have been implicated in the control of immune response function by experiments showing that anti-Ia alloantisera specifically inhibit such functions. Furthermore, the Ia structural genes are arranged so that the Ia molecules could provide a molecular explanation for Ir gene complementation effects. Since Ia antigens may have an active role in the control of immune response, these molecules must be analyzed to see if their structural features can explain how they carry out their proposed function.

The research projected here includes structural, genetic and serological investigation of Ia molecules. Structural analysis will include assessment of variability in primary structure, primarily by peptide mapping, localization of antigenic sites, search for interaction with other cell proteins, and mechanism of assembly and transport of Ia molecules to the cell surface. Genetic analysis will include a survey of available recombinants to further define the arrangement of Ia structural genes. Serological analysis will mainly involve a search for antibodies to unassembled Ia chains, or to all antigenically silent Ia molecules or chains.

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458. STRUCTURAL STUDIES OF IA ALLOANTIGENS

Silver, J., Hubert, J. J., Scripps Clinic & Research Foundation, Dept. of Cellular & Developmental Immunology, *476 Prospect St., La Jolla, California*, 92037, United States of America

The major histocompatibility complex (MHC) of the mouse controls a number of immunologically related functions. The immune response of individuals and inbred strains to a wide variety of synthetic and natural antigens is controlled by dominant genes that map within the I region of the MHC, and have been termed histocompatibility or H-linked Ir genes. The I region also codes for a series of cell surface (Ia) antigens. A number of experiments suggest that Ir genes exert their effect via cell interactions mediated by Ia alloantigenic molecules. With the goal of elucidating the molecular basis by which Ia alloantigens mediate their functions, we propose to subject them to detailed structural studies. We will employ microsequencing methods that have recently been developed, and which have previously been successfully applied to the structural analysis of the H-2K and H-2D gene products. Such studies may provide a framework for correlating the structure of these molecules with their functions.

459. MURINE IA MUTANTS - SOMATIC GENETIC AND BIOCHEMICAL STUDIES

McNicholas, J. M., Stanford University, School of Humanities & Sciences, Dept. of Biological Sciences, *Stanford, California*, 94305, United States of America

We propose a coordinate somatic genetic and biochemical approach to study a set of cell surface molecules coded by genes within the major histocompatibility complex, the Ia antigens, which appear to play a critical role in the regulation of the immune response. The mouse has been an excellent experimental system for studies of Ia antigens because of the availability of genetically defined inbred and recombinant inbred strains of mice, highly specific anti-Ia alloantisera, and more recently, monoclonal anti-Ia antibodies. The mouse and human major histocompatibility gene systems are similar and information concerning one system may be effectively applied to the other system. Therefore, these genetic and biochemical studies of the murine Ia antigens should contribute to the understanding of human Ia antigens. In particular in the human system, it will be important to understand the role these antigens play in HLA-associated predisposition to diseases, such as rheumatoid arthritis, multiple sclerosis and others. The first step in these studies is to obtain mutants from Ia antigen-bearing cell lines which lack or show abnormal expression of cell surface Ia. Mutant cell lines will be obtained by three different methods of cytotoxic immunoselection: 1) antibody and complement mediated; 2) cell-mediated; and 3) lectin resistant. The mutant cell lines will be characterized for cell surface expression of Ia by fluorescence activated cell sorter (FACS) analysis, genetically by complementation analysis, and biochemically by two-dimensional polyacrylamide gel (2-d PAGE) analysis of cellular proteins. From the analysis of Ia mutants with deficient or abnormal Ia chains, it is hoped that a more complete understanding of the genetics, structure, and expression of Ia antigens will be obtained which will facilitate further understanding of the complex functions of these cell surface molecules in immune responses and disease susceptibility.

460. SEROLOGICAL DEFINITION OF T CELL IA ANTIGENS

Trial, J., Washington University, School of Medicine, Dept. of Genetics, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

The summary for this project is under entry no. 271.

461. GENETICS AND FUNCTION OF MURINE IA ANTIGENS

Frelinger, J. A., Shigekawa, B., Woodward, J., University of Southern California, School of Medicine, Dept. of Microbiology, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

We are continuing our work on the immunobiology of the Ia bearing cells in the skin. We will further examine their kinetics of repopulation as a model for repopulation of macrophages in general. This will be examined directly as it has a significant impact on our studies on bone marrow chimeras and the role of H-2 in antigen presenting cells. We will further examine the cellular responses to minor histocompatability antigens and the role of H-2 in their presentation. These studies will be carried out through use of both biochemical and immunological approaches as well as genetic efforts.

BIBLIOGRAPHIC REFERENCES: Frelinger, J.A. and P.J. Wettstein. Expression of lymphocyte differentiation antigens on cells responsive to H-2 mutant alloantigens. J. Ret. Soc., in press, 1979. Frelinger, J.G., L. Hood, S. Hill and J.A. Frelinger. Epidermal Ia molecules of the mouse have a bone-marrow origin. Nature, in press, 1979.

462. INVOLVEMENT OF HLA ANTIGENS IN T CELL ACTIVATION

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OBJECTIVE: To study the involvement of 'self' HLA cell-membrane molecules in activation of T cells against different foreign antigens.

APPROACHES: Using in vitro tests with purified subpopulations of T cells and macrophages, and different antigens as well as antigen expressing target cells.

PROGRESS: We have shown that self HLA-D expressed in macrophages is co-recognized by sensitized T cells when activated by antigens such as PPD and hapten-conjugates. These important questions are now being investigated using antigens of direct relevance to cancer etiology.

463. IMMUNORESPONSE TO HUMAN SURFACE ANTIGENS

Croce, C. M., Botchan, P., Dolby, T., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

Mouse-human somatic cell hybrids containing either human chromosome 6, or 7, or 16, or 17, or 17 carrying the region p21 yields pter of human chromosome 6 as the only human chromosome present in the hybrids have already been produced and injected into syngeneic mice. Sera from hyperimmunized mice will be used to characterize the surface antigens coded by these human chromosomes and to further localize the position of the genes coding for these antigens on the respective human chromosomes. We will also determine whether the cellular immunoresponse to human histocompatibility antigens expressed in hybrid cells containing human chromosome 6 or part of human chromosome 6 is H2 restricted. The expression of H2 and HLA antigens will also be studied in hybrids between F9 mouse teratocarcinoma cells and either mouse cells derived from different mouse strains or human cells to determine whether in the undifferentiated hybrid cells the expression of H2 and HLA antigens is shut off and whether concordant expressions of mouse and human histocompatibility antigens occur in the differentiated hybrid cells. Syngeneic mice will also be hyperimmunized with somatic cell hybrids containing either chromosome 6 or the region p21 yields pter of human chromosome 6 and expressing HLA 3 and 7 or HLA 32, and their spleen cells will be fused with P3x63Ag8 mouse myeloma cells to obtain hybrids which produce antibodies against human histocompatibility antigens. This study should result in the selection of a series of hybrid clones producing monoclonal antibodies to different human histocompatibility antigens.

In addition, we intend to study the cellular and humoral immunoresponse to Epstein-Barr (EB) virus nuclear antigen (EBNA) and surface antigens using somatic cell hybrids between mouse cells and Burkitt's lymphoma cells.

464. MONOCLONAL ANTIBODIES TO HUMAN CELL SURFACE ANTIGENS

Ferrone, S., Molinaro, G., Quaranta, V., Glassy, M., Scripps Clinic & Research Foundation, Dept. of Molecular Immunology, *476 Prospect St., La Jolla, California*, 92037, United States of America

Using the hybridoma technique developed by Milstein et al., monoclonal antibodies have been prepared to human histocompatibility antigens. The specificity of these antibodies has been defined using serological and immunochemical tests. Some of the monoclonal antibodies recognize determinants which are different from those defining allotypic specificities, but have a restricted distribution on HLA molecules.

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465. IMMUNOBIOLOGY OF HLA AND ITS CLINICAL APPLICATIONS

Duquesnoy, R. J., Milwaukee Blood Center Inc., Dept. of Research, *763 N. 18th St., Milwaukee, Wisconsin*, 53233, United States of America

This project deals with the immunobiology of HLA and its clinical applications. Studies will focus on three areas. One is to identify and characterize B cell specific alloantisera, to detect DRw markers and antigens controlled by linked genes, and to characterize the role of such antigens in lymphocyte activation in the mixed leukocyte culture and in the secondary allogeneic response. We will also search for HLA-D homozygous typing cells with new specificities and determine the relationship between HLA-D and DR determinants. Studies of HLA and disease associations will concentrate on the complications of juvenile diabetes mellitus, thyroid neoplasia, acute lymphocytic leukemia, and ocular histoplasmosis.

BIBLIOGRAPHIC REFERENCES: Duquesnoy, R.J., Kozin, F. and Rodey, G.E.: High Prevalence of HLA-B27, CW1 and CW2 in Patients with Seronegative Spondyloarthritis. Tissue Antigens. 12:58-62, 1978. Stevenson, J.C. and Duquesnoy, R.J.: Distribution of HLA Antigens in Polish and German Populations in Milwaukee, Wisconsin. Am. J. Phys. Anthropol., in press 1978.

466. HUMAN IMMUNE RESPONSES TO HAPTENS IN VITRO

Rich, R. R., Baylor College of Medicine, School of Medicine, Dept. of Microbiology & Immunology, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The overall objective of this proposal is to develop a model for studying human immune responses to neoantigens on cell surfaces. To this end we will exploit techniques which we have recently developed for induction of human cell-mediated immune responses in vitro to hapten-conjugated cells. We plan to investigate both primary and secondary responses in a proliferative assay system. Most experiments will utilize trinitrophenyl-conjugated cells. However, dinitrophenyl and fluorescein conjugates will also be studied. The role of major histocompatibility complex genes and gene products in such responses will be of particular interest in two respects. First, we will investigate the possible role of HLA-associated Ir genes as determinants of responsiveness to hapten-conjugated cells. These studies will be performed in families, in an effort to associate high or low responsiveness with the inheritance of the HLA complex. Second, we will characterize the role of HLA-antigens in induction and elicitation of responses to hapten-conjugated cells. Stimulator and responder cell populations involved in these responses will be characterized functionally, physically and for cell surface properties. These studies should provide fundamental insights into T cell recognition and response in humans and the role of major histocompatibility genes and antigens in such responses. By providing a practicable in vitro model for evaluation of human immune responses to cell surface neoantigens, this work will also have important implications for studies of immune responses to neoplastic cells.

467. CELLULAR REGULATION OF DTH INDUCTION - EFFECT ON H-2 AND TUMOR-SPECIFIC ANTIGENS

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The aim of these studies is to gain an understanding of the effect of cellular regulation of the induction of DTH effector cells on allo-and tumor-specific antigens. It is hoped that such knowledge would facilitate elevation of the effectiveness of anti-TSA DTH responses in vivo.

The approach is based experimentally on an in vitro system for inducing DTH cells capable of producing a strong footpad swelling reaction against murine allo-antigens. The results of studies on the effect of regulation (positive and negative) of the induction of DTH on H-2 antigens will be applied to TSA in a syngeneic system. At a later stage, cells sensitized in vitro under conditions chosen to induce optimal anti-TSA DTH activity would be tested in vivo for their capacity to affect the growth of the mastocytoma P815.

468. THE CHEMICAL BASIS OF THE H-2 RESTRICTION ON CYTOTOXIC T CELLS

Cullen, S. E., Washington University, School of Medicine, Dept. of Microbiology & Immunology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

Recent reports indicate that the mouse H-2 complex plays a crucial role in cell-mediated cytolysis (CMC) directed against non-H-2 antigens. These reports demonstrate that histocompatibility between sensitizing and target cells at a portion of the H-2 complex is required for CMC in systems where the non-H-2 antigen is a viral, a minor histocompatibility, or a trinitrophenylated antigen. It has been suggested that this H-2 restriction mechanism may be relevant to in vivo CMC directed against neoplastic cells bearing a tumor associated antigen. To explain this histocompatibility restriction, the favored hypothesis pro-

posed in these reports is that the cytotoxic T cell recognizes 'altered-self'. The object of this proposal is to identify the chemical and/or structural basis of this alteration of self initially in the Sindbis virus system, and subsequently in a minor histocompatibility antigen system. A newly devised radioimmunoassay and cross-linking reagents have permitted the detection of an H-2 antigen-Sindbis antigen association. The nature of this association will be further explored with highly specific antisera to determine if H-2D and/or H-2K antigens are associated with the viral E1 and/or E2 antigens. These associations will also be confirmed by SDS-polyacrylamide gel electrophoresis and isoelectric focusing (IEF). Possible structural modifications of the H-2 antigen by Sindbis antigen (or vice versa) will be analyzed by comparison of IEF patterns, tryptic peptides and carbohydrate moieties derived from 'modified' and 'unmodified' antigens.

469. THE ROLE OF THE H-2L LOCUS ANTIGENS IN T-CELL MEDIATED CYTOTOXICITY

Levy, R. B., Hansen, T. H., Biddison, W. E., Shearer, G. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

BALB/c H-2db spleen cells generated a strong primary in vitro CML response against BALB/c (H-2)d wild type spleen cells. The effector cells generated were specific for L antigens. In an allogeneic CML response between two non-mutant strains directed against H-2D region antigens, two clones of cytotoxic effector cells could be identified, one with specificity for D antigens, and the second with specificity directed against L antigens. When syngeneic cytotoxic effector cells were generated against TNP modified H-2D antigens, no clones with specificity for L-TNP modified antigens could be detected. This contrasts with the H-2 restricted murine influenza model, in which influenza virus can be recognized by cytotoxic T cells in association with products of the L locus. Therefore, although products of the L locus are capable of inducing allogeneic cytotoxic effector cells, and serve as self recognition structures for influenza virus, these antigens appear not to be capable of eliciting syngeneic effector cells specific for the TNP hapten.

BIBLIOGRAPHIC REFERENCES: Levy, R. B. and Hansen, T. H.: Functional studies of the products of the H-2L locus. Immunogenetics 20: 7, 1980.

470. CELL MEDIATED LYSIS IN ARTIFICIAL LIPID VESICLES

DeGrella, R. F., Stanford University, School of Humanities & Sciences, Dept. of Chemistry, *Stanford, California*, 94305, United States of America

The objectives of this proposed research are to 1) prepare defined target vesicles containing purified H-2 and antigen that can be lysed by sensitized cytotoxic T cells, and 2) to use these vesicles to study H-2-antigen associations if any in these vesicles. Synthetic vesicles containing purified H-2 and coat protein (M-13CP) from the E. coli bacteriophage M-13 will be prepared by detergent dialysis methods. Antibodies to M-13CP and complement will be used to show that these vesicles can be lysed. Lymphocytes will then be sensitized to these targets and lysis of the target by cytotoxic T cells will be followed by 51Cr release, dye release, spin label release, and electron microscopy. Association of M-13CP and H-2 will be carried out using periodic pattern photobleaching.

471. GENETIC ANALYSIS OF THE PLASMA MEMBRANE IN MAN USING CELL HYBRIDS

Shows, T. B., Roswell Park Memorial Inst., Dept. of Experimental Biology, *666 Elm St., Buffalo, New York*, 14263, United States of America

A genetic analysis of the human cell surface will be accomplished at three levels: 1) external membrane proteins, 2) plasma membrane glycoproteins, and 3) membrane antigens. The external membrane proteins and glycoproteins will be labeled by standard procedures and analyzed by two-dimensional electrophoresis. Membrane antigens will be analyzed using the lymphocyte hybridoma strategy in which monoclonal antibodies are produced against human surface determinants. The expression of each of these three types of plasma membrane components will be ascertained on: 1) human cell lines derived from different tissues, 2) human cell lines from individuals with inherited metabolic diseases in which the plasma membrane may be altered, 3) transformed human cell lines, and 4) neoplastic human cell lines. The normal and abnormal electrophoretic and/or antigenic phenotypes determined for the plasma membrane components on these different cell types will be investigated in rodent-human cell hybrids. After fusion of the different human cell types with mouse cells the somatic cell hybrid methodology will permit dissecting the components of the plasma membrane and the determination of chromosome assignments of genes coding for human plasma membrane proteins, glycoproteins, and antigens. This research will characterize the plasma membrane phenotypes of a variety of different human cell types, determine if the phenotypes are of a genetic origin, and map human plasma membrane genes. Such information is important for understanding the genetic organization and biogenesis of the cell surface.

472. GENETIC CONTROL OF COMPLEMENT C4 AND LINKAGE TO HLA

O'Neill, G. J., Sloan Kettering Inst. for Cancer Research, Tissue Typing Laboratory, *410 E. 68th St., New York, New York*, 10021, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

473. CELLULAR ANTIGENS OF NORMAL AND MALIGNANT RAT TISSUES

Wettstein, P. J., Wilson, D., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

Major histocompatibility genes (MHG) code for cell membrane components involved in immune response regulation. The objective of this proposal is to refine our basic understanding of the genetics of these components and their phenotypic and functional expression in respect to immune differentiation.

Since it has become increasingly clear that all species, including man, display a general similarity with respect to their histocompatibility antigen system, genetically defined animal models are the most suited for investigations into the genetic fine structure, gene action regulation and evolution of this system, specifically in respect to the peculiarities of cell differentiation leading to immunocompetence and its deviations.

The major efforts of this proposal are: 1) To improve the possibility of investigating the genetic basis and functional aspects of these phenomena by continuation of production of specifically needed congenic rat lines; 2) the search for and selection of recombinants regarding cell membrane antigens and functionally recognizable traits such as mixed lymphocyte culture reaction, immune responsiveness, and susceptibility to disease; and 3) to elucidate the serological, biological, and molecular properties of gene products regulating immune responsiveness in rats and mice. Fifteen congenic lines are now in production: three taken over from the colony of Dr. J. Palm are nearly completed (N12) and others are in the second to fifth backcross generation.

474. GENETIC ASPECTS OF TUMOR-HOST IMMUNE RELATIONSHIP

Warner, N. L., University of New Mexico, School of Medicine, Depts. of Pathology/Medicine, *University Hill N.E., Albuquerque, New Mexico*, 87131, United States of America

The broad objectives of this project are to evaluate the influence of genetic controls on the tumor-host immunological relationship - both at the level of the cell surface antigens that can provoke such tumor responses, and at the level of host genetic control operating in the various mechanisms of 'immune' recognition. These studies include: (1) An analysis of the expression of various H-2 and I region antigens on a variety of tumor cell types; (2) an analysis of the expression of Hh antigens on normal and tumor cells, and of the nature of the genetic control of host responses to Hh antigens; (3) analysis of the natural killer cell reactive with certain tumor cells and its possible role in immunosurveillance; (4) determination of the possible role of MHC gene products in tumor specific antigens on lymphoid and plasma cell tumors; and (5) analysis of possible Ir genetic control of T cell mediated immunity against various tumor associated antigens.

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475. H-2 ANTIGEN EXPRESSION IN CULTURED MOUSE LEUKEMIA CELLS

Rajan, T. V., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Depts. of Pathology/Genetics, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

The summary for this project is under entry no. 235.

476. STUDIES ON H-2 MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) MUTANTS (STRUCTURAL ANALYSIS AS A PROBE OF FUNCTION-STRUCTURE RELATIONSHIPS)

Nathenson, S. G., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Depts. of Microbiology & Immunology/Cell Biology, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

The H-2 major histocompatibility complex (MHC) is a complex of genes vital to the system of immune recognition and reaction. Studies are proposed to determine the structural basis of the mutation in the K or D genes of the H-2 MHC which are responsible for histogenic and, in some cases, serological reactivity with the parent strains. Analysis of the mutant products will be carried out by tryptic peptide mapping to determine if differences exist. Localization of tryptic peptide differences will be made to specific CNBr fragments in order to localize the mutational site within the molecules. Sequence analysis from selected mutants will determine the nature of the molecular alteration of the mutation.

B. OTHER ANTIGENS OF THE HISTOCOMPATIBILITY COMPLEXES

477. RELATION OF BLOOD GROUP AND HUMAN TUMOR ANTIGEN

Hakomori, S., Watanabe, K., Rupar, C. A., Nudelman, E., Wang, S., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

Membrane-bound blood group determinants have been characterized as being carried by sets of glycosphingolipids (e.g., Aa, Ab, Ac; H1, H2, H3, etc.) and by carbohydrate chains bound to the hydrophobic membrane proteins such as 'band 3' protein. Alterations in the structure of the carrier portion as well as the terminal determinants are associated with development and with cancer. Specifically, we have detected a progressive branching of the carrier carbohydrate chain during development of erythrocytes. In human cancer, two types of significant changes in blood group determinants have been noticed; namely, the appearance in human cancer of incompatible blood group antigens foreign to the host, and the incomplete synthesis of blood group determinants and the associated accumulation of precursor chains.

This application is to propose studies on: 1. Characterization of structure in detail and variation of the membrane-bound blood group ABH, Lewis, Ii and Forssman antigens and their carrier molecular species. Particular focus will be made on the determinants carried by gangliosides, polyglycosylceramides and hydrophobic membrane protein ('band 3' or alike). 2. Further studies on ontogenetic changes in blood group carrier carbohydrate chains and a possible change of Forssman antigens during ontogenesis of endodermal organs. 3. Chemical and immunological characterization of the 'incompatible' blood group or Forssman isoantigens in human cancer and their corresponding isoantibodies present in normal and patients' sera. Particular emphasis will be the characterization of A-like antigen in tumors of O or B, and further extensive studies on Forssman antigen in tumors of F(negative) individuals in various cancers. 4. Enzymatic basis for synthesis of 'incompatible' blood group and Forssman isoantigens and for the process of i to I or I to i conversion.

BIBLIOGRAPHIC REFERENCES: Hakomori, S., and Young, W.W. Jr. Tumor-associated glycolipid antigens and modified blood group antigens. Scandinavian J. Immunol. Supplement 6, 97-117, 1978. Feizi, T., Childs, R., Hakomori, S. and Powell, M.E. Blood group Ii-ganglioside of human erythrocyte membrane. Biochemical J. 173, 245-254, 1978.

478. ABO BLOOD GROUPS AND CARCINOEMBRYONIC ANTIGEN IN COLONIC CANCER

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The summary for this project is under entry no. 8.

479. TISSUE BLOOD GROUP ANTIGENS AND CARCINOGENESIS

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No summary has been provided to the Smithsonian Science Information Exchange.

480. IDENTIFICATION OF CELL TYPES RESPONSIBLE FOR REJECTION OF GRAFTS BEARING WEAK ANTIGENS

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OBJECTIVE: To determine the cell types responsible for rejection of grafts bearing weak antigens.

APPROACH: Weak transplantation antigens are thought to mimic tumor antigens. We have, therefore, explored immune responses to a weak histocompatability antigen, the male specific antigen, H-Y. We need to determine which factors influence these responses in the hope that this information will be applicable to the control of responses to tumor antigens, and that better ways of manipulating anti-tumor responses in vivo can thus be devised.

PROGRESS: Two main T cell responses to H-Y antigen have been found: (1) The generation of H-2 restricted cytotoxic responses in vitro and (2) the rejection of grafts in vivo. The genetics of response (1) are very complex, and imply control mediated via antigens of the major histocompatability complex (MHC) both in mouse and in man. The genetics of response (2) are less complex but it is controlled by MHC genes and it seems clear from studies with H-2 congenic and recombinant mice that response (1) and response (2) are mediated by different cell types. We know at least the identity of the effector cell in the in vitro response (1), but there is scant information about the effector cell type(s) involved in graft rejection.

481. BEHAVIOR OF WEAK TRANSPLANTATION ANTIGENS

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Primary attention is being devoted to the behavior of grafts which are only incompatible with respect to weak transplantation antigens in mice and rats. Under certain conditions these grafts can evoke an immune response, while under other conditions they may induce unresponsiveness. The basis for these different responses is being studied. More specifically the projects include investigating the behavior of H-Y incompatible grafts (of various types, e.g., skin, parathyroid, islets of Langerhans), as well as those incompatible for putative skin-specific antigens. Studies are also aimed at determining the genetic basis for the expression of, and response to, H-Y antigen in rats. Finally, some attention is also being devoted to elucidating the mode of action of some coat color determinants of the mouse.

BIBLIOGRAPHIC REFERENCES: Berryman, P.L. and Silvers, W.K. 1979. Failure to confirm the influence of splenectomy on the survival of H-Y incompatible skin grafts in mice. Transplantation 27:287-288. Silvers, W.K. and Collins, N.H. 1979. Silvers, W.K. and Collins, N.H. 1979. Behavior of H-Y incompatible neonatal skin grafts in rats. Transplantation 28:57-59.

482. IMMUNOBIOLOGY OF THE H-Y (MALE) ANTIGENIC SYSTEM

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The long-term goals of this proposed research are to use the well-defined H-Y antigenic system to: (1) Study the regula-

tion of the immune response against H-Y; (2) study the genetic regulation of the expression of H-Y antigen; and (3) further advance the field of immunogenetics of spermatozoa. The in vitro serological detection of the H-Y antigen on the surface of mouse spermatozoa and male epidermal cells, with the cytotoxic test, permitted the further analysis of the H-Y system as to humoral and cellular immune factors involved in H-Y incompatibility. Our specific aims are to gain insight into such questions as: (1) What role do suppressor cells or factors play in the (a) long-term survival of male skin grafts on females of certain strains of mice which nevertheless produce cytotoxic antibody, and (b) in the specific unresponsiveness to male skin rejection by multiparous females? (2) Determine whether or not the antigenic representation on the sperm surface is coded by the haploid genome of the sperm or the diploid complement of its precursor cells.

In a broader context, in as much as H-Y incompatibility is now seen to be a system in which graft survival occurs despite an immune response (antibody production), it provides a parallel with the growth of antigenic tumors in hosts that produce antibody and therefore has relevance to cancer as well as to transplantation biology.

C. RELATIONSHIP BETWEEN HISTOCOMPATIBILITY ANTIGENS AND TUMOR ANTIGENS

483. STUDIES ON TRANSPLANTATION ANTIGENS AND OTHER CELL SURFACE ANTIGENS

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OBJECTIVE: The classical histocompatibility antigens (HLA-A, B, C, H-2K, D, etc.) are highly polymorphic cell surface components which constitute the target molecules recognized by T-killer lymphocytes in graft rejections. We have earlier shown that these classical transplantation antigens share structural features with immunoglobulins. One of the main objectives of the present study is to investigate if the transplantation antigens are immunoglobulin-like also functionally.

APPROACH: We have clearly shown that a virus-coded protein in adenovirus-infected cell lines is associated with transplantation antigens on the cell surface. We intend to continue these studies in order to elucidate the nature of that molecular interaction. Moreover, we want to investigate if the transplantation antigen virus protein is recognized by T-killer cells in order to obtain a molecular explanation of the immunological rejection of tumor cells. We also plan to investigate the primary structure of the transplantation antigens in order to clarify if the genetic polymorphism is located in clusters in the molecule or scattered throughout the whole structure. We would like to correlate these findings with the existence of hyper-variable regions in immunoglobulins.

Studies of transplantation antigen-like molecules which are expressed early during the autogenesis will be continued. We plan to isolate these molecules in chemical amounts in order to try to elucidate in detail their structural similarities to the classical transplantation antigens.

484. NEOPLASIA AND HISTOCOMPATIBILITY ANTIGENS IN SERUM

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One major objective is the use of monoclonal antibodies to assess the structural relationship between histocompatibility and tumor associated antigens on murine tumor cells and to examine possible H-2 viral interactions. Efforts will also be made to further chracterize the immunological and molecular profiles of H-2 and Ia antigens with cross reactive anti-HLA antibodies. The other major objective of our studies is to continue our ongoing efforts to delineate the cellular and molecular heterogeneity of H-2 and Ia antigen on murine cells with monoclonal antibodies.

BIBLIOGRAPHIC REFERENCES: Callahan, G.N., Pellegrino, M.A., Ferrone, S., Haubrich, L. and Reisfeld, R.A. Expression of a sheep red blood receptor by a murine lymphoma. J. Immunol. 123:2725-2728, 1979.

485. RELATION OF HLA ANTIGENS AND LYMPHOCYTE FUNCTION TESTS TO TUMOR SUSCEPTIBILITY AND CLINICAL COURSE IN E. AFRICAN BL AND NPC (ABBREV)

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OBJECTIVE: (a) To determine whether there is any link between certain alleles of the major histocompatibility complex (particularly of the HLA DR locus) and susceptibility to Epstein-Barr virus (EBV), related tumors in East African subjects. (b) To measure blood lymphocyte responses to a range of antigenic and mitogenic stimuli in serial specimens from the same donors.

Answers to (a) may help to identify individuals at high risk of developing these tumors, and may provide some clues as to the nature of the interaction between EBV and the target cell. The object of (b) is to seek some definition, in quantitative terms, of the clinical phenomenon of 'host response' to these tumors.

APPROACH: (a) HLA typing at the A, B, C and D loci will be carried out on cryopreserved lymphocytes from at least 75 East African patients with Burkitt's lymphoma (BL), 80 first order relatives (consisting of families in which multiple tumors have occurred and families of individual BL patients where specimens from both parents and siblings are available), at least 75 nasopharyngeal carcinoma (NPC) patients, and matched control subjects (including families) from the same geographical area will be examined. Standard tissue typing sera will be used as well as a panel of sera derived from East African women subsequent to pregnancy. (b) A new microassay developed by Dr. S. C. Knight and extensively validated on cryopreserved lymphocytes from the I.A.R.C. cell bank makes it possible to measure the response of cells from a single ampoule to a range of antigenic or mitogenic stimuli. These include phytohaemagglutinin (PHA) and mitomycin C blocked cells from lines such as U266BL (derived from human myeloma) and DAUDI (from BL).

486. HLA ANTIGENS AND SURVIVAL IN MAMMARY CARCINOMA PATIENTS

Pellegris, G., Muscolino, G., Bertario, L., Costa, A., Illeni, M. T., Leo, E., Saccozzi, R., Istituto Nazionale per lo Studio e la Cura dei Tumori, Immunoematology Laboratory, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: The aim of this work is to verify HLA typing as to whether immunogenetic factors may be suspected as playing any role in conditioning the survival of patients for over 8 years after surgical removal of mammary carcinoma.

APPROACH: Two groups of patients will be typed for HLA A,B and C antigen: one group showing relapse and/or metastasis more than 8 years after surgical removal of the tumor, the other being apparently free of tumor clinical signs. Each group will include 30 to 50 patients untreated by chemo- and/or hormonotherapy.

The lymphocytotoxic test will be used.

Controls will be healthy unrelated females, similar to the patients as regards age, and living in the same geographical area as the patients.

487. HLA AND GENETIC MARKERS FOR TERATOCARCINOMA

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The objective of this proposal is to investigate the significantly increased HLA-Dw7 antigen frequency in patients with testicular teratocarcinoma. This will be done by critically analyzing and reconstructing the triangular relationship between the mouse major histocompatibility complex (H-2), the T/t complex, and teratocarcinoma, and then applying this information, as a guide to look at genetic regions in linkage with HLA in hopes of defining genetic markers in association with teratocarcinoma. Experiments will be designed comparing a testicular teratocarcinoma population to a normal control population from the same geographical area in terms of: 1) Genetic markers in linkage with HLA (CLO, PGM3, Bf, C2 and HLA-DR) and 2) genetic markers expressed on spermatozoa. Because T/t com-

plex alleles are expressed only on spermatozoa as the only normal adult tissue in mice and control the expression of a teratocarcinoma associated antigen on sperm (F9), a systematic investigation will be made for a T/t complex-like region near HLA (in the human), controlling the expression of genetic markers present on spermatozoa of teratocarcinoma patients. Considering the homology between the mouse and human major histocompatibility complex (H-2 and HLA), such a region in the human should be included within the same genetic areas as the other markers, i.e. between HLA-D and the centromere. The detection of spermatozoa-specific teratocarcinoma associated antigens will involve three different techniques: 1) Lymphocyte-spermatozoa primed lymphocyte typing (PLT); 2) two-dimensional protein electrophoresis of various components of spermatozoa and 3) screening of sera (i.e., HLA alloantisera, sera from teratocarcinoma patients) for anti-spermatozoa activity. We expect that the structural, genetic, and developmental association between teratocarcinoma, and T/t complex, and H-2 in the mouse, will help unravel the relationship between HLA-Dw7 and teratocarcinoma as well as provide an effort toward the definition of a T/t complex-like region in the human.

488. FORSSMAN ANTIGEN/ANTIBODY AND HUMAN ADENOCARCINOMA

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The overall goal of this project is to investigate the relationship between the globoside-Forssman isoantigenic system and the occurrence of various types of adenocarcinoma in humans in order to determine if there is a basis for attempting specific immunotherapy of some cancer patients using Forssman (Fs) antigen. In this project, tumor and normal tissue will be obtained from patients with gastric, colon or breast adenocarcinoma. The tissue will be lyophilized and will then be tested by antibody absorption in order to determine if the tumor and or the normal tissue contains Fs antigen. In addition, sera from patients with different stages of disease will be tested for Fs antibody in order to determine if there is a relationship between the level of Fs antibody and tumor recurrence or metastases. The class of Fs antibody (i.e., IgM and/or IgG), in healthy people and in adenocarcinoma patients with various stages of disease, will also be determined. In addition, we will test by antibody absorption the white cells of normal individuals for the presence of Fs antigen. If Fs antigen is present on white cells, the test will enable the Fs antigen status of humans to be readily determined and will enable us to confirm the existence of a reciprocal relationship between Fs antibody and Fs antigen. Since the Fs antigen status of the tumor and the level of Fs antibody in the serum may be affected by the formation of immune complexes, we will determine if circulation immune complexes (IC) are present in the sera of patients with adenocarcinoma. If ICs are found, they will be characterized and the complement status of the patients will be determined.

489. PHYSICAL RELATIONSHIP OF THE H-2 ANTIGENS AND RNA-TUMOR VIRUS

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OBJECTIVE: The tumorigenesis of some RNA-tumor viruses depends on the H-2 genotype of the host. Moreover recognition of both H-2 and virus antigens appears to be necessary for the occurrence of T-cell cytolysis. Therefore, it is important to know whether host cell surface H-2 antigens are incorporated into the virions as the host cell membrane becomes part of the complete particle during the budding process.

APPROACH: With the indirect immunoferritin technique the localization of H-2 antigens on mouse mammary tumor virus (MMTV) and murine leukemia virus (MuLV) will be studied. In this project the following systems will be examined: a) MMTV- and MuLV-producing cells with different H-2 genotype, b) Cells with same H-2 genotype infected with different strains of MMTV and MuLV, c) Localization of the H-2 regions: K, D and L on MMTV and MuLV.

PROGRESS: BALB/c mammary tumor cells (H-2d genotype susceptible for MMTV infections) infected with MMTV from C3H were incubated with antisera against H-2d followed by incubation with ferritin-labeled anti-mouse IgG. The cell surface was labeled and the virus particles were either labeled or unlabeled. This suggests that in susceptible cells the virus does not select a budding place in relation to the H-2 antigen.

490. BIOCHEMICAL CHARACTERIZATION OF ALIEN HISTOCOMPATIBILITY ANTIGENS EXPRESSED ON MURINE FIBROSARCOMA CELLS

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OBJECTIVE: To compare the peptide composition of alien and original H-2 molecules expressed on methylcholanthrene-induced fibrosarcoma cells to the peptide composition of H-2 molecules expressed on normal cells. The knowledge of the biochemical characteristics of alien H-2 molecules could provide an important information on the genetic mechanism responsible for the expression of inappropriate determinants on tumor cells.

APPROACH: Normal and alien molecules, metabolically labelled by short-term in vitro culture of tumor or normal cells in presence of radioisotopically labelled precursors, will be purified by immunoprecipitation with specific antisera, followed by chromatography on SDS-PAGE. The purified H-2 molecules will be subjected to treatment with proteolitic enzymes. The resulting peptides will be characterized by ion-exchange chromatography. The peptidic maps of alien antigens and of the corresponding determinants on normal cells will be compared with each other to determine similarity in the primary structures of the molecules.

491. INDUCTION, GROWTH AND REPRESSION OF PRIMARY AND TRANSPLANTABLE NEOPLASMS (HOST AND TUMOR CELL VARIABLES) (ABBREV)

Law, L. W., Appella, E., Rogers, M., DeLeo, A., Old, L. J., Hopkins, N., Alaba, O., Parmiani, G., DuBois, G., Chang, K. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Biology Laboratory, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

OBJECTIVES: Major emphasis is placed upon the study of tumor antigens of the transplantation rejection type (TSTA), and of tumor antigens (TA) assayed by in vitro techniques and of the immune responses they evoke. As a corollary to this study the biologic properties in vitro and in vivo of histocompatibility (H-2) antigens are under study. Solubilization and methods of purification of both TSTA and H-2 are under investigation with the ultimate purpose of defining these membrane antigens after purification in physico-chemical, biologic and molecular terms. The role of a B-tropic retrovirus as a vector in the transmission of genetic information of a specific tumor antigen (TSTA of Meth A) is now under study as well as the use of TSTA markers (Meth A) in the hope of defining more clearly the molecular events in transfection studies using DNA of chemically induced sarcomas.

BIBLIOGRAPHIC REFERENCES: Law, L.W., DuBois, G.C., Rogers, M.J., Appella, E., Pierotti, M.A., and Parmiani, G.: Tumor rejection activity of antigens isolated from the membranes of a methylcholanthrene-induced sarcoma, C-1, bearing alien H-2 antigens. Transplantation Proceedings XII: 46-49, 1980. DeLeo, A.B., Jay, G., Appella, E., DuBois, G.C., Law, L.W., and Old, L.J.: Cell surface antigens of chemically induced sarcomas of inbred mice. Transplantation Proceedings XII: 65-69, 1980.

492. CROSS-LINKING OF HISTOCOMPATIBILITY AND TUMOR ANTIGENS

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The existence of tumor specific antigens has been known for many years; however, antigens which are recognized by syngeneic cytotoxic T cells have not been isolated and chemically characterized. One reason for this difficulty may be MHC restriction, wherein both the tumor antigen and the major histocompatibility antigen of the tumor cell are recognized by cytotoxic T cells. The altered self hypothesis proposes a physical association between tumor and MHC antigens to form an altered antigen.

We propose to look for complex formation between tumor and MHC antigens using chemical crosslinking of surface antigens and to analyze the resulting complexes using specific anti-

body and polyacrylamide gel electrophoresis. The ability of crosslinked complexes to inhibit cytotoxic T cell activity will be tested. The model system employed will be vesicular stomatitis virus infected congenic mouse 3T3 fibroblasts. Further studies will involve crosslinking of mouse myeloma tumor cells.

493. CELL SURFACE ANTIGENS OF MURINE TUMORS

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The aim of this proposal is to investigate the biological and chemical profile of murine major histocompatibility (MHC) antigens (H-2 and Ia) on neoplastic cells. Since it has been demonstrated that there are alterations of H-2 and Ia antigens on tumor cells, a concerted effort will be made to characterize the expression of these MHC antigens. In addition the biochemical makeup of both MHC antigens and tumor associated antigens (TAA), as well as their relationship to one another, on tumor cells will be determined and compared with that of MHC antigens on normal cells. Attempts will be made to assess the capacity of these antigens to elicit cellular and humoral immune responses in order to evaluate the effect of malignant transformation on these cell surface markers. Xenoantibodies to tumor associated antigens will be used to develop sensitive radioimmunoassays for TAA and to compare the cross-reactivity of TAA from various tumors. Circulating TAA will also be biochemically and immunologically characterized in order to evaluate their role in the pathology of malignant disease. In addition, the appearance and quantity relationship to circulating MHC antigens will be determined and correlated with stages and locations of tumor growth. A study of quantitative changes in the expression of MHC antigens on tumor cells, as well as their relationship with neoantigens of the tumor, will provide information essential to an assessment of the role of these antigens in cell communication and control phenomena. In addition a precise determination of the time course of appearance of altered and neo-antigens both on the cell surface and in the serum will allow for a critical evaluation of the potential of these changes for immunodiagnosis and immunotherapy.

BIBLIOGRAPHIC REFERENCES: Callahan, G.N., Pellegrino, M.A., and Walker, L.E. Alterations in expression of glycoproteins by a murine fibrosarcoma. Transplant. Proc. XII, 87, 1980. Ferrone, S., Pellegrino, M.A., and Callahan, G.N. Human and murine tumors: Changes in cell surface structures coded by the major histocompatibility complex region. J. Immunogenetics 7, 71, 1980.

494. CELL-SURFACE ANTIGENS OF MURINE TUMORS

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Malignant transformation is accompanied by extensive cell surface alterations. Of particular interest are alterations of histocompatibility antigens, cell surface components which have been implicated in cell-cell interactions in the immune response and in histogenesis. This project will employ serological and immunochemical techniques to characterize cell surface antigens of a series of spontaneous and chemically induced murine tumors. The antigenic profiles of the tumor cells will be analyzed by 2-dimensional electrophoresis of immunoprecipitates. Specific antisera will be used to assess the contribution of fetal and viral antigens to the total antigenic mosaic. Histocompatibility antigens expressed by tumor cells will be subjected to extensive immunochemical analysis in order to detect structural alterations. Immunofluorescence techniques and bivalent crosslinking reagents will be used to examine topographic relationhips between histocompatibility and tumor-associated antigens on the cell surface. Serum from animals bearing tumors will be examined for the presence of tumor associated and histocompatibility antigens. These studies will contribute to our understanding of the structural basis of altered-self recognition in relation to tumor immunity and should provide insight into the role of cell surface structures in regulation of cell proliferation and positional control.

495. RELATIONSHIPS BETWEEN HISTOCOMPATIBILITY ANTIGENS AND TUMOR SPECIFIC TRANSPLANTATION ANTIGENS OF METHYLCHOLANTHRENE-INDUCED SARCOMAS

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The summary for this project is under entry no. 65.

496. HUMAN TUMOR ANTIGENS - RELATIONSHIP OF TUMOR ANTIGENS AND TRANSPLANTATION ANTIGENS

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The rejection of tumor grafts between animals suggests that tumor antigens operationally can behave as transplantation antigens. This, coupled with other recent evidence, suggests that tumor antigens may in fact represent a modified histocompatibility antigens. Our objective has been to demonstrate a possible structural similarity between tumor and histocompatibility antigens. It was hypothesized that if such a relationship exists, beta-2-microglobulin, the species-specific low molecular weight chain common to the histocompatibility antigen of all species, would be similarly expressed in the putative tumor antigen. Affinity chromatography employing anti-human beta-2-microglobulin antiserum was used to purify both the HLA and putative HLA-like tumor antigen from normal and cancerous tissues. Using an in vitro assay for tumor antigen activity (leukocyte adherence inhibition, LAI), we found that material purified by this affinity chromatography method possessed tumor antigen activity. The material so purified demonstrated an HLA-like subunit structure on DSD-polyacrylamide gels. Presently, antitumor antibody is being prepared using the monoclonal antibody method.

497. IMMUNE RESPONSE TO MODIFIED SELF AND TUMOR ANTIGENS

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The nature and control of the immune response to modified self cell surface antigens will be investigated as a model for the anti-autochthonous tumor response. Our approach is to use syngeneic cellular carriers, and their isolated membrane antigens, as tolerogenic carriers capable of inducing B cell unresponsiveness. We will analyze the nature of the optimal carriers and the mechanism of tolerance in this system as a prelude to using tumor-specific antigens (TSAs) as haptens. Furthermore, since hapten-modified syngeneic cells induce a cytotoxic T-cell response to haptenated H-2 (in the mouse), we will study the parameters of this response, its involvement (if any) in the suppression of the B cell response, and possible ways of augmenting the generation of cytotoxic T cells. This will be primarily effected by the associative recognition of allogeneic histocompatibility antigens on the modified cells. Finally, we will apply the knowledge gained in these areas to the regulation of the immune response to TSAs and the immunotherapy of human neoplasia.

498. ADJUVANT EFFECT OF ALLOGENEIC HISTOCOMPATIBILITY ANTIGENS IN IMMUNOLOGICAL REACTION AGAINST TUMOR-ASSOCIATED ANTIGENS

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OBJECTIVE: To test, for therapeutic purposes, the possibility of increasing the host response to weak tumor-associated antigens by means of allogeneic histocompatibility antigens carried by the same cell.

APPROACH: Our previous finding on rat skin homograft survival (Experientia, 30, 947-949, 1974) and very recent data (Fed. Proc., 37, 2052-2054, 1978) on hamsters have shown a clear adjuvant effect. We aim to detect such a phenomenon in in vivo and in vitro systems using allogeneic SV40-, M-MSV-, and RSV-transformed cells, as well as mouse lymphosarcoma and embryo cells as sensitizers. The parameters studied are: tumor growth, host survival and in vitro cytotoxicity of spleen cells.

PROGRESS: In vitro cytotoxicity (3H-proline test) against syngeneic SV40-transformed (KB/cSV) cells was evaluated in

BALB/c mice after immunization with either KB/cSV and KD2SV cells. (The latter, obtained from SV40-transformed cells of B10.D2 mice, show the same MHC (H-2d) while presenting a different pattern of minor histocompatibility antigens.) A higher cytotoxicity was not observed when immunizing with KD2SV cells. Thus, an adjuvant effect by minor histocompatibility antigens in reaction against antigens associated with the transformation by SV40 has not been evidenced in this experimental design. After immunization with KB/cSV cells, a cytotoxicity higher than that observed using KD2SV cells as immunogen was frequently found; the possible relationship between this finding and age of the animal is under investigation.

499. HISTOCOMPATIBILITY ANTIGENS IN DEFINED MEMBRANES

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The overall objective is to define the chemical and physical parameters which regulate immune cellular recognition. Plasma membrane vesicles obtained from tumor target cells are being characterized for enzyme activities, extent of glycosylation of H-2 proteins, asymmetric orientation, and interaction with specific alloantibodies. We also plan to purify the histocompatibility antigens by isoelectric focusing and reconstitute them into defined lipid systems which mimic the environment of the naturally occurring lipids in the plasma membrane. We will test these reconstituted antigens for the ability to inhibit recognition between a specific cytotoxic T cell and its target cell or to inhibit target cell killing.

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